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ORIGINAL ARTICLES

THE PRINCIPLES INVOLVED IN THE ECONOMIC READJUSTMENT OF DIETARIES*

BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO.

THE basic standard of diet is its energy value. The energy value of the foodstuffs is the currency of dietetics. Just as in the barter and trade of commerce some common basis of exchange is necessary, something, that is to say, having a standard value with which the value of all other things can be compared, and by the use of which they can be bought and sold; so with food, in order to exchange one foodstuff for another in the dietary, some standard of relative values—some food currency—must be used. The basis of monetary currency is the dollar; that of food is the calorie.

This does not necessarily mean that the calorie, although itself a fixed value, is always of exactly the same dietetic importance, for just as in the monetary system the purchasing power of the gold dollar may vary in different countries, so in dietetics may the relative importance of the calorie vary with the foodstuff which supplies it. Thus, the ration of one individual may be quite inadequate or may be harmful for another. "Ae man's meat's anither man's poison." Quite apart from gratification of appetite, which, however, is a most important factor in food assimilation, the food consumed by each individual must be properly adjusted to meet his peculiar requirements. Besides its caloric value, therefore, certain other values for foods must be taken into consideration, but at the outset attention is called to the calorie.

A calorie is the unit of energy. It can be used to measure the expenditure of energy, whether this occurs as heat, or as mechanical work, or as electrical discharge, or as chemical reaction. To make this plain, consider the relationship between the expenditure of energy as motion and as heat in the case of a

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steam engine. When the engine is at rest, all the energy is dissipated as heat, but when it works, some of this heat takes the form of movement, the sum total of energy expenditure being the same whether the engine is standing still or is moving. The source of the energy given out by the engine is obviously the fuel which is burned in the furnace. Turning now to the animal body, the fuel which it burns is the food. Everything capable of burning outside the body is not suitable for the animal fuel, but only those substances which can be acted on by special types of reagents present in the animal, called enzymes or ferments, which have the function of loosening up and breaking apart into smaller molecules the large complex molecules of which food is composed. The smaller molecules then unite with the oxygen of the blood and become burned or oxidized and liberate their energy. The foods belong to three classes of organic substances: fats, carbohydrates, and proteins.

In order to find out how much energy is available in a fuel, it is necessary to burn it and measure the amount of heat which it gives out in the process. The measurement of the amount of heat is not, however, so very easy a matter to comprehend. It is not a question merely of increase in temperature, for it is obvious that there must be very different *amounts* of heat in an ounce and in a pint of boiling water; placed side by side in similar vessels the ounce of water will cool off much more quickly than the pint. This simple example indicates a basis upon which we may measure heat, namely, as the temperature multiplied by the volume or mass of water. The unit of such measurement is called a calorie, which is defined as the amount of heat required to raise the temperature of 1 kg. of water (2¼ lb.) through 1° C., or, roughly, 1 lb. H_2O, 4°F. In order to measure the caloric value of a fuel or foodstuff, we must, therefore, ascertain the degrees of temperature through which a known volume of water is raised by burning a weighed amount of substance in such a way that all the heat which it gives out is taken up by the water.

The apparatus for doing this is called a calorimeter, which consists, in principle, of a stout iron chamber, or bomb, into which a weighed amount of the fuel, or food, is placed and then filled with compressed oxygen. The bomb is tightly closed, placed in an outer vessel containing a known volume of water at a known temperature, and the fuel or food set on fire by an electric current. When 1 gm. (15 gr.) quantities of each of the three foodstuffs are burned in the calorimeter, the values are: protein, about 5.5; fat, 9.3; carbohydrate, 4.1. That means, in the case of fat, for example, that if 1 gm. be burned in the chamber of a calorimeter, which, it will be assumed, contains 1000 gr. water, an increase of temperature amounting to 9.3° C. will be observed. No matter how slowly or quickly the given material burns, the caloric value is always the same.

To compute the total energy taken into the body, all we have to do is to weigh each article of food, ascertain from the numerous dietary tables readily available in textbooks, encyclopedias, government bulletins, etc., how much fat, protein and carbohydrate it contains, and then multiply each of these by its proper caloric value and add the results together. This gives us the caloric value available when the foodstuffs are completely burned. But some of the food is not entirely oxidized in the animal body, so that to determine the physiologic caloric value, we must subtract from the crude value the caloric

value of the unused portion given out with the excreta. In the case of fats and most carbohydrates, the subtraction is very small if digestion and assimilation be in good order. It is considerable, however, in the case of protein, bringing the actual caloric value of this foodstuff down to 4.1, the same as for carbohydrates.

So far we have gone on the assumption that the caloric value of the foods will be the same whether they are quickly burned or only slowly used up in the life processes of the animal body. Obviously, however, before dietetics can be considered to rest on any scientific basis, indisputable evidence that such is really the case, must be furnished. The energy output of animals must be measured to show that it balances up exactly with the energy value of the food which has meanwhile been consumed. One of the greatest accomplishments of modern physiologic science is the fact that it should have been possible to do this very thing. For this purpose a calorimeter is again used, but modified so that it may be constantly ventilated to prevent suffocation of the animal. This is called a respiration calorimeter. It consists of an air-tight chamber with double walls, in the space between which is an ingenious device by which the temperature of the air of the space is regulated so as to be exactly the same as that of the chamber itself, thus preventing entirely any loss of heat. The heat given off by an animal placed in the calorimeter is measured by observing the change in temperature produced in a known volume of water passed through radiators in the chamber, that which is meanwhile employed to evaporate the water given out in the expired air and sweat being readily computed by collecting this vapor in suitable absorption bottles (see p. 746) placed in the course of the ventilating tubes. Calorimeters have been constructed by Atwater, Benedict and Graham Lusk, in which a man can live comfortably for long periods of time.

The above comparison can not be made by merely measuring the food the animal eats, because the food is not necessarily consumed immediately after it is taken; it must first of all become assimilated in the body, and this takes some time. Months indeed may elapse between the time that a food is eaten and that when it is ultimately oxidized to yield energy. It is on this account that a starving animal may go on yielding energy; he lives on the foodstuffs which have been incorporated with his body; he feeds on his own tissues. The problem is not so simple as in the case of a steam engine, where we may compare the energy output with the fuel consumption.

How, then, is it known that each of the foodstuffs gives out as heat and other forms of energy the same amount of heat which it produces when burned in a calorimeter? The rate at which combustion is proceeding in the body must be measured by measuring the products of the combustion.

To understand properly the principle upon which such a measurement depends, turn for a moment to the conditions obtaining when a piece of sugar or fat is burned outside the body. As the combustion proceeds, carbonic acid and water are given off because of the union of the oxygen of the air with the carbon and hydrogen, of which elements, along with some intramolecular oxygen, either of these foodstuffs is composed. By the oxidation, energy is liberated as heat, so that the number of calories given out by the burning process

is directly proportional to the amount of water and carbon dioxide meanwhile produced and the amount of oxygen used up. It is, chemically, a comparatively easy matter to measure the amounts of these combustion products. The carbonic acid is measured by finding how much alkali it can change into carbonate and by leading the air which contains it as vapor through substances, such as concentrated H_2SO_4, which absorb it. The analysis of proteins is more complicated on account of the fact that, besides carbon, hydrogen, and oxygen, they contain nitrogen. By suitable chemical processes this nitrogen has either to be dislodged from them, or converted into its hydrogen compound, ammonia, which is then very easily measured.

Let us see now how these methods of the chemist may be employed to measure the products of combustion in the case of an animal. For this purpose the chamber of the calorimeter is connected with an air-tight system of wide-bore tubes, along which an air current is made to circulate by means of a rotary blower or fan. The water and carbon dioxide given off by the animal are caught in so-called absorption bottles, inserted in the system and containing suitable reagents to combine with the gases. The oxygen consumed by the process of combustion causes the volume of air in the system to shrink, but just as quickly as it does so, as indicated by a gauge, fresh oxygen is discharged into the system from a cylinder of the gas. In a chemical analysis, as we stated above, the water value, as well as that of carbon dioxide, is used in calculating the composition of the substance burned. In a metabolism experiment, however, as the above physiologic method is called, the water excretion is not of much value as a means of determining the amount of combustion, because of the fact that relatively large and inconstant amounts of it are taken with the food, and the body at different times contains varying amounts of it. The carbon dioxide excretion, taken along with the oxygen intake, is the important criterion. In short, then, by measuring the carbon dioxide absorbed by the absorption bottles and the fresh oxygen that has to be delivered into the system to keep the volume constant, one can tell exactly how much material containing carbon and hydrogen is being oxidized in the body.

When we know how much carbon has been oxidized, we can not tell how much of it came from protein, fat, or carbohydrate, because all three contain it; and the energy value of each being different, we can not compute how many calories have *in toto* been liberated by the combustion process. To find out what foodstuff was actually consumed, some other excretory product that is peculiar to one or the other of the foodstuffs must be observed. In the case of protein, this is made possible by the fact that the amount of nitrogen which the animal excretes during his stay in the chamber can readily be measured. Having found from the nitrogen excretion how much protein must have become used up (by multiplying grams of nitrogen by 6.25), we may then calculate the amount of carbon contained in this amount of protein and substract it from the total carbon which must have been burnt to produce the carbon dioxide. The remainder is the carbon of the fat and carbohydrate that have been burnt. The relative amounts of these two can then be computed from a knowledge of the relationship of the oxygen absorption to the carbon dioxide excretion—the so-called respiratory quotient.

Having thus become acquainted with the general principles of which the necessary measurements are made, the results are next to be considered. These are among the greatest achievements of modern medical science, for when the measurements are properly made, the caloric output as directly measured corresponds exactly with that calculated by multiplying the amount of each foodstuff, known to have been burned in the body, by its caloric value. This accurate correspondence of the *direct* and *indirect* methods of calorimetry has both a practical and a theoretic interest. It shows, for one thing, that there can be no energy either created or lost in the animal body that is not accounted for by the oxidation of the foodstuffs. It shows that no energy can be absorbed from the outside or new forms of energy created in the animal body. It proves the law of the conservation of energy for the animal mechanism.

And now with regard to the application of these extremely important facts in the science of *dietetics*. We are in a position to determine with scientific accuracy just exactly *how much food should be taken under varying conditions of bodily activity*. In a general way, we know that the amount of food that is required is proportional to the nature and amount of bodily exercise that is being performed at the time, and that, if the food supply is inadequate, the work before long will fall off, not only in quantity, but in quality as well. "Horses (and men) work best when they are well fed, and feed best when they are well worked," is an old adage and one the truth of which cannot be overestimated in the consideration of all questions of dietary requirements. An ill-fed beggar will rather suffer from the pain and misery of starvation than attempt to perform the piece of work that the well-meaning housewife bargains should be done before she gives him the meal. The spirit may be willing but the flesh weak. If he could be trusted, he should be fed first and worked afterwards. Besides the amount of work, two other factors are well known to influence the demand for food, namely, growth and climate. A young growing boy will often demand as much if not more food than would appear, from a comparison of his body weight with that of his seniors, to be his proper share, and, other things being equal, it is well known that we are inclined to partake much more heartily of food during the cold days of winter than during the sultry days of July and August.

That we know these facts, in a general way, indicates that the first steps in the exact determination of dietetic requirements must be to find out how much energy the body expends under varying conditions of activity, etc. It must be plain from what has already been said that this may be done by having the person live for some time in a respiration calorimeter, so that we may measure the caloric output by both the direct and the indirect methods, the results of the one serving as a check on those of the other. To the conclusions drawn from results of observations made under such artificial and unusual conditions of living, the objection might, quite justly, be raised that they need not apply to persons going about their ordinary routine of life. To meet this objection another method, which we may call the *statistical*, is available. This consists in taking the average diet of a large number of individuals and comparing its caloric value with the average amount and type of work that they are meanwhile called upon to perform. This can be done in cases where the diet is accurately known, as in public institutions, the army, the navy, etc. The total food supplied is then divided

by the number of individuals, this giving the *per capita* consumption. Obviously some get more than others, but when a sufficient number of individuals is included, such errors become eliminated by the law of averages. The close agreement between the results secured by these two quite different methods is a guarantee of the reliability of either.

Before proceeding to consider the results in greater detail, it is plain that, in order to make it possible to compare the energy output of individuals of different sizes, the results, that is, the caloric output, must be determined for some standard size of body. If such a standard were not used but we merely quoted the results as so many calories given out during each hour or day, we should, of course, find that a small child gave out far less energy than a large full grown man; we should fail entirely to bring out the fact, which is of great significance, that relatively to the size of their bodies the child gives out considerably more energy than the adult. The standard usually employed is that of body weight, the kilogram (2¼ lb.) being the unit. During recent years, however, it has been claimed that a more accurate basis of comparison is the unit of body surface, the square meter; but with the reasons for this change of standards and the controversial matters which have been raised in connection with them, we need not concern ourselves; for the purposes of a scientific study of the principles which govern dietary requirements, the body-weight standard is the most suitable to adopt at present.

Let us then consider the caloric output of a healthy man of average weight (70 kg.). In the first place, it must be measured while he is at perfect bodily rest, lying quietly in bed, and at such an interval of time after taking food that the digestive organs are inactive. This is done by having him sleep in a bed placed in the respiration calorimeter and measuring the caloric output the first thing in the morning when he awakes. Under such conditions it has been found that 1 C. per kg. per hour, or $1 \times 70 \times 24 = 1680$ C. per diem, is produced. At first sight this result would seem to indicate that food containing an amount of protein, fat and carbohydrate capable of yielding 1680 C. would meet the daily requirements, but such is not the case. More must be given to allow for the fact that the physiologic processes involved in the assimilation of food by the tissues, quite apart from anything else, causes some heat to be dissipated from the body. This property of food has been clumsily called its *specific dynamic action*, and it varies according to the nature of the foods, being largest in the case of protein and smallest in the case of carbohydrate. As a rough estimate, it is usually considered that the average daily diet has a specific dynamic value amounting to ten per cent of the resting caloric output, giving us therefore, for a resting man living on an average diet, a daily caloric output of $1680 + 186 = 1848$ C.

The further expenditure of calories depends entirely on the amount of muscular work done, and much interesting information has already been collected, showing just how many calories are set free in the performance of different types of work. It is significant that, with their far-sighted appreciation of the value of science in the welfare of the state, the German authorities should some years ago have appropriated considerable sums of money for just such investigations, and that the data should have served as one of their main guides in the apportionment of food to the people. The government, as soon as it saw

the possibility of a shortage, placed the control of food in the hands of scientific food experts without permitting such a vital question to be trifled with by legislators whose knowledge of food values is as shallow as their knowledge of how to play obstructive politics is profound. The delay that has attended the passage through Congress of the bill for food control is all the more provoking when we consider that in our country, and ready to serve voluntarily, are men who have convincingly shown themselves to be second to none in the world, not only in the scientific knowledge which must guide, but also in the executive ability which must administer, an efficient food control.

It has been found that if the person instead of lying in bed be made to sit still in a chair in the calorimeter, his caloric output increases by about 8 per cent, and if besides this he be made to do such work as writing at a desk, it will increase by nearly 30 per cent. A little simple arithmetic thus shows us that for such work the caloric expenditure per hour while the man is doing such work will increase from 77, which we saw to be that of basal heat production and the specific dynamic action of the food, to 97 C.; and if he does such work for sixteen hours and sleeps eight hours, this gives $8 \times 77 = 616 + 16 \times 97 = 1552 = 2168$ C. A great increase in caloric output is created by walking, even on the level, and it has been quite clearly shown that the difference between the value which we have arrived at, viz., 2170 C., and the actual caloric expenditure of 2500 C., which from statistical studies is known to be expended by individuals doing a light day's work, can readily be accounted for by the walking incidental to moving from place to place in the daily routine.

Coming now to the results of the statistical method, the reliability of this method is testified to by the remarkable correspondence in the caloric values of the food consumed by farmers in widely different communities:

	Calories
Farmers in Connecticut,	3,410
" " Vermont,	3,635
" " New York,	3,785
" " Mexico,	3,435
" " Italy,	3,565
" " Finland,	3.474
Average,	3,551

(Lusk: The Fundamental Basis of Nutrition)

The average inhabitant of various cities:

London,	2,665
Paris,	2.903
Munich,	3,014
Königsberg,	2,394
	(Rubner)

Individuals in different callings:

Farmers' families (U. S. A.),	3,560
Mechanics' " "	3,605
Professional men's families (U. S. A.),	3.530
Army (U. S. A.),	3,851
Navy "	4,998
	(Atwater)

In general it is usually computed that a man weighing 70 kg. requires in calories:

2,500 for a sedentery life.
3,000 for light muscular work.
3,500 for medium muscular work.
4,000 and upwards for very heavy toil.

(McKillop)

These figures apply to the average man, but in calculating the caloric requirements of a family or a community allowance must be made for the lesser requirements of women and children. Several dieticians have compiled tables showing how many calories are expended according to age and sex, and the German authorities have recently taken these figures from them and calculated a generalized mean, which shows in comparison with men the percentage that should be allowed for women and children. The figures are as follows:

Man,	100
Woman,	83
Boy over 16,	92
Boy, 14-16,	81
Girl, 14-16,	74
Child, 10-13,	64
Child, 6-9,	49
Child, 2-5,	36
Child, under 2,	23

(McKillop)

In calculating the caloric requirement of the population as a whole, the necessity of making allowance for the varying needs of men, women and children would obviously make the calculations far too complicated for practical purposes. It is necessary to have a factor by which we may multiply the total population in order to determine its *man value*. This factor is based on the relative proportion of men to women and children, and it amounts very nearly to 0.75; i. e., three quarters of the total population gives "the man value." Knowing the total population, say, of a city, we must therefore multiply this by 0.75 in order to ascertain for how many men doing moderate muscular work (3000 C.) food has to be provided.

Although the first step in estimating the dietary requirements of a family or community is thus to ascertain how many calories are expended by each individual and then to find suitable foodstuffs that will supply this amount, it must not be imagined that we have thereby fulfilled all the conditions to be considered in drawing up a correct dietary. There are many other factors to consider, and these, for simplicity's sake, we may divide into two groups: first, those pertaining to the chemical nature of the foodstuff, and secondly, those pertaining to its palatability, digestibility, and availability.

To appreciate the importance of the *chemical nature of foods*, it will be well to return to the analogy of the animal body with a steam engine, not because we shall find that the analogy becomes any closer, but, because it so entirely breaks down in one important particular that it becomes of value on this very account. The fuel of the engine is fuel alone; it is used for no other purpose, whereas the food of an animal, besides being fuel, is also used to repair the tis-

sues of the body which have become broken down on account of the constant wear and tear to which they are subjected in carrying on the processes of life. The next step is, therefore, to find out the relative importance of the foodstuffs in supplying material for the reparative processes in the tissues. Biochemical investigation has shown that these are composed of the same classes of chemical substances as the foods—proteins, fats, carbohydrates, salts, and water—and that proteins occupy the most important position, since the greater part of the living tissue is composed of them; namely, the cell and its nucleus. Some fats or fat-like substances are also associated with protein in the construction of this vital tissue machinery, but by far the greatest bulk of the fat found present in the body merely represents storage fuel deposited, not in, but between the really active or vital tissues. Some carbohydrate is also probably used to construct the machinery, but by far the greater proportion is used for fuel purposes; indeed, carbohydrate is the most readily available of all the foodstuffs for purposes of producing energy. There is no large store of carbohydrate in the body, because it is quickly consumed, whereas fat may be stored away for some considerable time before it is ultimately used as fuel material. Standing distinctly apart from the others in dietetic importance, therefore, is protein. With this foodstuff alone, many animals can exist, although they may not thrive, whereas with fats and carbohydrates as the sole foodstuffs, life is impossible. Protein in the diet is a *sine qua non* of life, because it is more than a mere fuel: it is also the essential building material for the worn out tissues.

This unique position of proteins has, in a general way, been appreciated for many years, but, apart from the fact that, of all foodstuffs, proteins alone contain nitrogen, so little was known concerning their chemical structure that, with the exception of gelatin, all proteins were thought to be of much the same dietetic value. Thanks to the advancement of biochemical knowledge, it is now known that this view is very far from being correct, for proteins differ in their chemical structure and in their dietetic value. The differences are dependent upon the nature and proportions of the various groups of smaller molecules of which the highly complex and very large molecule of protein itself is composed. To make this clear, let us imagine the protein molecule as a completed building with its stone and lime, its woodwork and plumbing, its plaster, and so on. It is composed of a great variety of building materials; but in a row of buildings no two need be exactly alike (although the same materials in general are used in their construction); some of them may have no stonework, others no plumbing, and even in those which use all of the available materials, the relative quantities used will vary considerably. So with protein: it is built up of innumerable building materials belonging to the class of chemical substances known as amino acids, that is, organic acids whose acidity is practically neutralized by the inclusion in the molecule of an ammonia residue, called the amino group. There is a great variety of such amino acids, some comparatively simple and others highly complex, since they contain, besides the organic and amino group, other chemical groups that are often of highly intricate structure.

To know the name and structure of each of these protein building materials, or amino acids, is not necessary for our purpose here, but there are two or three that we must at least mention in order to be, later on, in a position to

understand why certain proteins should be more valuable than others as foodstuffs. These are lysin, containing two ammonia groups; tyrosin and tryptophan, containing a so-called aromatic group; and cystin, containing sulphur. The protein of muscle, for example, is not composed of exactly the same variety and proportion of amino acids as that of egg white. Even the proteins of the same tissues of different animals may not be exactly the same in their amino-acid consistence. It is evident, then, that if, on account of wear and tear, the tissues should require certain amino acids with which to reconstruct their protein, the supply can be insured only provided the food contains proteins yielding these particular amino acids. In the process of digestion the proteins become broken down into the amino acids, which are then absorbed into the blood. The most perfect protein food would thus be one containing all of the amino acids found present in the proteins of all the tissues, for then each tissue could select from the blood the exact amount of each of the amino acids it required, and what one tissue did not require the others might make use of. In this manner all of the amino acids of a protein foodstuff might theoretically be used as building material for worn out tissue protein; but such a perfect adjustment between supply and demand does not actually occur, there being always a surplus of some amino acids, just as there would surely be some building material unused from a wagon, initially filled with every variety, after it had supplied to each one of a row of houses the materials required for repair purposes. This rejected building material has to be got rid of from the body, and this is accomplished by the amino acid being split up into two parts, of which the one is burned to yield energy, and the other, consisting of ammonia, is excreted in the urine as urea.

The proteins that contain all of the essential amino acids, though in varying proportions, are those of animal origin, such as the casein of milk and the albumin and globulin of blood, eggs, and muscle. Certain vegetable proteins, such as are present in part at least in the soy bean, hemp seed, Brazil nut, maize, and wheat (glutenin), also contain all of the necessary amino acids, though not in such suitable proportions as in proteins of animal origin. These may be designated as vegetable proteins of the first quality. Other vegetable proteins, such as those of beans, peas (legumin), part of the protein of maize (zein) and wheat (gliadin), etc., on the other hand, are wanting in one or more essential amino acids and may be designated as of second quality.

These facts have been ascertained by actual chemical analysis of the proteins, and their relationship to the building up of tissue proteins has been demonstrated by observing the rate at which young animals grow when fed on different proteins. During growth it is plain that the building-up process in the tissues is occurring in exaggerated form, so that by observing the weights of the animals from day to day the rate of the process can be measured. It is no doubt the case that proteins which are inadequate for growth, will also be inadequate for the repair of broken-down tissues in the adult. By taking a piece of paper ruled in squares and placing the weights of the animals on the horizontal lines and the days of observation on the vertical, we obtain what is known as *the curve of growth.*

Many of the earliest observations were made on young rats and mice. To eliminate individual errors large numbers of the animals were used, all of them being fed on a uniform diet of carbohydrates, fats, and salts, to which

was then added the particular protein whose influence on growth it was desired to investigate. Large numbers of animals were used in each group, so as to eliminate individual peculiarities and accidental errors. Fed on the basal diet alone, the animals did not live for more than a few days. If protein of animal origin, such as the casein of milk or the albumin of milk or egg, or blood were added, however, the curve of growth was exactly like that of a normal animal. The proportion of protein that had to be given to attain normal growth varied considerably in different animals. In the case of white mice 25 per cent of the total calories had to be given as protein; rats required 15 per cent, and man seems to need only 7 per cent, this being the proportion in human milk on which alone the human infant thrives. With vegetable proteins, such as the glutenin of wheat and maize (Indian corn), which contain all the amino acids, normal growth could also be secured, but more of the protein had to be given than was the case with animal proteins, because some of the amino acids are not present in adequate amounts. With vegetable proteins in which certain amino acids were missing, however, the animals did not grow at all. Thus, with one of the proteins of maize known as zein, the curve of growth actually descended, showing that the animals must soon have died of starvation. Chemical analysis shows that two essential amino acids are wanting in zein, namely, lysin and tryptophan. By adding pure tryptophan along with the zein, a distinct improvement was noted in the curve; it no longer descended, but remained practically horizontal, indicating that now, although incapable of growing, the animals were being at least maintained. Evidently, then, proteins must contain tryptophan if they are to prevent starvation. If lysin, as well as tryptophan, were given along with the zein, the curve of growth became normal, that is, it became the same as that obtained when perfect proteins such as casein are fed. Lysin, therefore, must be an important amino acid for growth, and it is of great significance that there is a high percentage of lysin in all those proteins that are concerned in nature with the growth of young animals; thus, it is present in large amount in casein, lactalbumin and egg vitellin.

The condition of the animal that has been fed on inadequate proteins is of great interest. When the aromatic amino acid, trytophan, which, as we have seen, is essential even for maintenance, was absent, the animal soon passed into a serious condition of malnutrition. Its fur became ruffled, its eyes inflamed, its feet cold, and it remained in a condition of torpor. By adding tryptophan to the diet, these symptoms immediately disappeared, and the animal became perfectly normal in every respect except that it failed to grow. It remained healthy but stunted. A most interesting question here presents itself, namely, has the power to grow become lost, or has it merely become suppressed by the absence of lysin? It is of great significance that growth merely becomes suppressed, for when the stunted animal was given a perfect protein, such as casein, it immediately began to grow with great rapidity, and soon attained the size of its now full-grown brothers and sisters.

Although it is particularly the vegetable proteins that are likely to be deficient in essential building stones, certain animal proteins, such as gelatin, are also lacking. Gelatin, like zein, contains no tryptophan, and like zein, it can not,

therefore, maintain life. It is not a true protein but it is a valuable adjunct to protein food, because it contains many useful amino acids.

It should be emphasized that in wheat and maize besides the imperfect proteins, gliadin and zein, there is also another protein that is of first quality, namely, glutenin. This is present in sufficient amount to make even strictly vegetarian diets perfectly safe, provided enough of either of these cereals is taken to allow for the fact that only a part of the protein is of first quality.

Now it will be asked, how are we to make certain that suitable variety of protein building stones is present in the diet? The answer is that there is little chance of inadequacy in this regard provided several varieties of protein food are given. "Provide sufficient calories and let the proteins take care of themselves," is a perfectly safe rule to work by, provided animal proteins are used. Real danger from protein starvation could arise only in the case of strict vegetarians who did not take a sufficiency of wheat or corn, for, although other vegetable proteins than glutenin do contain all of the essential amino acids, yet they may be deficient in this regard, and it would be decidedly risky to attempt to live on them alone. Strict vegetarians are, therefore, liable to run the risk of partial starvation. One of the most valuable of proteins is probably casein of milk; another vitellin of egg yolk. A glass or two of milk with an egg, along with vegetable food, makes the diet a safe one, provided always, of course, that the caloric requirements are met, and that no excessive wear and tear of the tissues is going on.

But it is probable that such a diet is inferior to one containing a proper, but not excessive, amount of animal proteins. It has been found that the smallest amount of protein required to maintain nutritional equilibrium is secured by taking flesh food, along with abundance of carbohydrate and fat, because obviously this, in its amino acid make-up, comes closest to that of the animal's tissues.

These considerations lead to the question: To what extent may the proportion of protein in the diet be reduced with safety? It is evident that there must be a minimum below which every one of the necessary building materials of protein would not be supplied in adequate amount to reconstruct the worn-out tissue protein.

The extent to which the protein content of the diet of man can be lowered with safety depends on several factors, of which the most important are: first, the nature of the protein; secondly, the number of nonprotein calories; and thirdly, the extent of tissue activity. Where so many factors must be taken into consideration, the only method by which the actual minimum can be determined consists in what may be called "cut and try" experiments. Of the many investigations of such a nature, probably the best one is that recently published from the Nutrition Laboratory of Copenhagen. The subject, an intelligent laboratory servant, lived a perfectly normal and active life for a period of five months on a diet of potatoes cooked with margarine and a little onion, and containing 4000 C., with a total protein content of 29 grams. During another period he did outdoor work as a mason and laborer, and took 5000 C. daily, and 35 grams protein. Many other experiments of a similar nature make it certain that man can lead a normal existence and remain in good health

on very much less protein than the 100 grams which statistical studies show to be the amount which he actually takes. This discrepancy between the amount which experiment demonstrates to be adequate and that which habit and custom demand, raises the question as to whether, after all, our instincts may not have erred and so made us unnecessarily extravagant in our protein intake. It has been suggested that such protein extravagances, will in various ways, have a deleterious effect on the organism; thus, that the excretory organs, such as the kidneys, will be overtaxed in eliminating the unused amino acids, that the constant presence of the bodies in excess in the blood will cause degeneration and sluggish metabolism, and that the excess protein in the intestine will lead to the production of ptomaines, whose subsequent absorption into the blood will cause toxemic symptoms.

Important support to such views appeared to be supplied some dozen years ago by Chittenden, who was able to show that he himself and many other persons doing different kinds of work could be supported on daily amounts of protein that were not more than from one-third to one-half of the amount usually taken. Not only so, but it was averred that distinct improvement was experienced in the general sense of well-being and of mental efficiency as a result of the lesser protein consumption.

Taking these results as a whole, it is quite clear that man can get along under ordinary conditions with much less protein than he ordinarily takes. This really proves nothing, for the question is not *can* he, but *should* he so deprive himself. Are instincts and customs wrong, and is Chittenden right?. That is the question. To answer it many studies have been made of the condition of peoples who for economic or other reasons are compelled to live on less protein than the average. Are these people healthier, less prone to infections and degenerative diseases, and more efficient mentally than others? In such studies great care must be exercised to see that conditions other than diet, such as climate, exercise, etc., are properly controlled. It would not, for example, be fair to compare the mental and bodily condition of peoples living in the tropics and who take comparatively little protein, with those living in temperate zones, who consume much more. After discounting all of these other factors, it has been quite clearly shown that, when the protein allowance is *materially reduced*, the people as a whole are less robust, mentally inferior and, instead of being less prone to the very diseases which are usually supposed to be due to overloading of the organism with useless excretory products, they are more liable to suffer from them. That a considerable reduction in protein weakens the defense of the organism against infection is probably due to the fact that the fluids of the body normally contain a great variety of so-called antibodies, that is, of highly complex substances that are largely protein in nature. When bacteria, or the poisons produced by them, enter the body, they are met by one or more of these defense substances and destroyed or neutralized. Now it is clear that there should always be a surplus of protein building materials from which these antibodies may be constructed. Such an excess will constitute a "factor of safety" against disease. And there are factors of safety of another nature to be produced, two of which we are in a position to appreciate. In the first place, there must always be an adequate supply of

tryptophan, of lysin, and of cystin, not only to meet the bare necessities of the protein constructive processes that go on under normal conditions, but also to make good the larger amount of protein wear and tear that greater degrees of tissue activity will entail. Although moderate muscular exercise does not appear to cause any immediate consumption of protein (carbohydrate and, later, fat being the fuel material used to produce it), yet it does throw a greater strain on the tissues, a greater wear and tear of the machinery, and hence a demand for more protein building material. In the second place, there are certain of the internal secretions of the body, such as epinephrin (adrenalin), which are essential for life, and as crude materials for the manufacture of which certain amino acids are essential. Tyrosin is one of these, and since, as we have seen, proteins differ from one another quite considerably in the amount of this amino acid which they contain, it is advisable to provide an excess so that an adequate supply of tyrosin may always be available.

The answer to one of the most important practical questions in dietetics, namely, "What proportion of protein should the diet contain?" depends on these scientific principles. The source of the protein is the important thing. With animal protein there is no doubt that we could get along with perfect safety by taking daily not more than 50 to 60 grams, which is about half of what we actually take. If the protein be of vegetable origin and of the first quality, such as is contained in wheat and maize preparations, more should be taken, because of the fact that the protein in these cereals is only half of the first quality. When vegetable proteins of the second quality, such as those of peas, beans, lentils, etc., are alone available, much larger amounts are necessary. Such proteins are inadequate in the case of growing children at least, and even in adults it is undoubtedly advisable that other proteins should supplement them.

To insure safety, therefore, it is almost imperative that the diet should contain *proteins of various sources.* If for economic reasons the main source must be proteins of vegetable origin, then some animal protein, such as is contained in milk or meat or eggs, should be added to at least one of the daily meals. Thus, when peas and beans are mainly depended on for the protein supply, they should be taken either with milk or one of its preparations, or with a thick gravy or sauce made from meat and containing the finely minced meat. This must not be strained off, for if it is, the sauce will contain only the meat extractives but not any of the protein, for these are coagulated by the boiling water. Meat extract, in other words, contains no proteins; it is not a food but merely a condiment of no greater dietetic value than tea or coffee.

The question has been asked, "What should we take in place of meat if one or two meatless days have to be introduced in order to conserve the food supply?" The answer is that milk and eggs will completely make good the deficiency, or if these also be unavailable, then the taking of a more liberal supply of wheat or maize preparations will be satisfactory. Protein deficiency for one or two days a week could, however, scarcely entail any risk to health provided the usual allowance of animal protein or of first quality vegetable protein be allowed on the other days. The value of potato protein should be remembered in this connection. In any case the attempt should always be made

to give a variety of proteins. That the animal economy prefers, if it does not demand, such a mixture even if the best varieties of protein is indicated by the fact that milk, the perfect food for early growth and development, contains two such proteins—casein and albumin.

Regarding the relative quantities of fats and carbohydrates, the usually accepted figures are: fat, 80 grams (3 oz.); carbohydrates, 400 grams (14 oz.); that is, a ratio of 1 to 5.

Even when the calories and the protein are correct, the diet may be inefficient because of absence of minute quantities of peculiar substances of unknown composition. These have been called vitamines, but this is a most unfortunate name, since an amine is a well characterized chemical substance, whereas these "accessory food factors," as they are better called, are not. Failure of nutrition due to the absence of accessory food factors is really no recent discovery. It was known to sailors in the bygone days of sailing vessels that despite a liberal allowance of well-mixed preserved food, a long voyage was almost certain to lead to the development of ill health, despondency, and incapacity for work, or perhaps of the disease, scurvy, itself. The discovery was, however, made by the famous Captian Cook that this unhealthy condition of his sailors could be relieved by compelling them whenever possible to go ashore and eat of the fresh foods, either animal or vegetable, that might be available. It was perfectly clear that such foods contained something of great benefit to health that was lacking in the ship's galley. The giving of orange or lemon juice in certain cases of malnutrition in children has also been known for some time in medical practice, but the impetus to a more searching investigation of the nature of these unknown accessory food factors was given by the discovery that the curious disease called beriberi, often observed in certain tropical countries, was associated with the taking of polished rice in place of the less popular grain still having some of the husk attached. This observation led to a systematic investigation of the association between this and the analogous disease which develops in pigeons when these birds are fed exclusively on polished rice. It is found that the addition to the polished rice of an alcoholic extract of the husks very promptly removed the symptoms, and that other things like yeast had a similar effect. Several investigators attempted to isolate this vitamine, as they called it, in pure condition, and thus determine its exact chemical composition, but with little success. Among the most careful of these investigations are those of McCollum, who has come to the conclusion that there are at least two accessory factors concerned, one of them soluble in fat and present in adequate amount in butter and other animal fats, but not in vegetable oils, and the other soluble in water and present in wheat, vegetables, fruits, etc. Milk contains both of these factors, so that its inclusion in a diet is a safeguard, not only against inadequacy in suitable protein, but also against the absence of accessory food factors. There is little danger of the diet being inadequate with regard to food factors if it contain some fruits or green vegetables or unheated fresh milk. The food factors are destroyed by prolonged cooking.

Digestibility and Palatability.—No matter how perfect in calories, protein,

and accessory food factors a diet may be, it will fall short of being really adequate if it is not properly assimilated. It is the function of the digestive apparatus to break up the highly complex molecules of protein, fat, and carbohydrate sufficiently to permit them to pass through the lining membrane of the intestine into the blood and lymph. This disintegration is effected by the digestive ferments which are contained in the digestive juices. Of these, saliva, the gastric juice, and the pancreatic juice are best known. In the cases of protein and carbohydrate, the activities of the ferments are interdependent, in the sense that the foodstuff must be acted on by the ferments in a definite order. One ferment prepares the foodstuff for the action of the next. Without this preliminary treatment the second ferment can not properly unfold its action. The conditions are like those existing in a factory where the products of one department are further worked up in another, which then hands on its product to a third and so on.

These facts indicate that for efficient digestion it is essential that the *initial* digestive juice be secreted in proper amount and at the proper time. The first digestive ferment which acts on protein is the pepsin of gastric juice. A fundamental question in dietetics is: On what does the secretion of this juice depend? The answer is: On the gratification of appetite. No one, judging from his own experience, will probably deny the correctness of this answer, for we all know that unappetizing food is likely to be followed by a sense of gastric discomfort, if not by symptoms of indigestion. Are there, however, any scientific observations from which the true value of this factor in the initiation of the digestive process can be appraised? Thanks to the brilliant work of the great Russian physiologist, Pavlov, we have such information. Pavlov's experiments were made on dogs, but the results have been shown, particularly by Carlson, to be very similar in the case of man. The observations were made on animals in which an artificial opening or fistula had been made into the stomach. Through this fistula the secretion of gastric juice could be observed, and it was found that very shortly after taking some savory food by the mouth, a copious secretion of gastric juice was set up, and that this occurred even although the food after being swallowed was prevented from entering the stomach, by making a fistula in the esophagus. The experiment is called "sham feeding." Not only this, but a hungry animal would secrete the gastric juice even although the food was not actually placed in the mouth but merely offered to it. The anticipation of an appetizing meal, as well as the gratification of receiving it, can set up the flow. It is called the psychic or *appetite* juice. If the animal were not hungry or had no appetite for the particular food, no juice was observed to flow.

The pepsin in this psychic juice sets the ball of protein digestion rolling. Once started, this process goes on automatically, because the digestive products produced by the appetite juice have the power of directly stimulating the gastric glands to further activity, and when the food has been digested to a somewhat further stage, the stomach delivers its contents, in small quantities at a time, into the beginning of the intestine, where by again acting directly on the lining membrane it excites the flow of pancreatic juice, a ferment of which, namely, *trypsin*, carries the digestion to still another stage, until finally the

protein molecule is sufficiently broken up to be attacked by the ferment *crepsin* present in the intestinal juice and intestinal mucosa. The whole process, therefore, depends for its proper accomplishment on the appetite juice. It is like a fire: the psychic juice is the kindling material; when it is ignited, the combustion goes on automatically, one stage leading to the next. Some substances, such as the so-called extractives of meat, act like partially digested protein in directly stimulating the secretion of gastric juice.

We have seen that practical dietetics depends on several factors, the exact relative importance of which can not perhaps in every case be gauged, but preparation of the food so as to make it appetizing, must undoubtedly rank high. The importance of *good cooking* will now be apparent. It is the act of making food appetizing and, therefore, digestible. It is really the first stage in digestion, the stage that we can control and one therefore to which much attention must be given, especially when it becomes necessary to make attractive articles of diet ordinarily considered common and cheap. Most people can cook a beefsteak or a lamb chop so as to make it reasonably appetizing, but few can take the cheaper cuts of meat and convert them into cooked dishes that are as popular and attractive. There are still fewer who can take the left-overs and trimmings and convert them in the same way. This is the real art of cooking, and too much encouragement can not be given to the effort which our cooking experts are making to show people how these things can be done. The waste of good food in a large city is appalling. An army could live off our garbage cans. I need not dwell on this most important phase of the food conservation problem. I would only add that every housewife who desires to do her "bit" in the present emergency can do so in no way better than by learning to use *all* the odds and ends of the kitchen in such a way that they can be offered as appetizing food to her household. It is worse than useless to dish things up unattractively, for under such circumstances food becomes poison.

Cooking has other advantages than making the food appetizing. The heat loosens the muscle fibers of the meat so that it is more readily masticated; it destroys microorganisms and parasites in the meat; it destroys antibodies which might interfere with the action of the digestive ferments. Thus, raw white of egg is not digested in the stomach because it contains one of the antibodies which prevent the pepsin from acting on it. Boiled egg white, if properly chewed, is digested, and whipping the egg white into foam partly destroys the inhibiting substance.

Before concluding, something should be said about the *laxative qualities of food*, for it is often in this particular alone that one food is more satisfactory than another. A diet of meat, milk, eggs, and white bread is apt to be unphysiologic because there is nothing in it to serve as what has been called intestinal ballast, that is, a material which will keep the intestines sufficiently filled to stimulate their muscular movements. This ballast is best furnished in the shape of cellulose, the most important constituent of green food. Peas, beans, cabbage, salad, and many fruits, especially apples, should always occupy a place in the daily menu. Another valuable food yielding this ballast is the outer grain of wheat, oats, etc. So much must not be taken as to produce a constant intestinal irritation, and each person must determine for himself where

this limit lies. The difference between various breads is almost entirely in the degree to which they supply ballast.

APPENDIX.

It will be remarked that nothing is said in the foregoing article concerning the methods by which a given diet can be composed so as to supply the required number of calories. This is a detail which could be adequately discussed only by reference to extensive diet tables, the publication of which is unnecessary here. It may help if we give some rough and ready rules by which such tables may be satisfactorily used. As a type of diet table take the following:

FOOD	PROTEIN PER CENT	FAT PER CENT	CARBOHYDRATE PER CENT	CALORIES PER LB.
Average of beef, veal, and mutton	14.5	16.1	...	913
Pork	12	29.8	...	1477
Bacon	9.5	59.4	...	2685
Fish (general average)	10.9	2.4	...	295
Eggs (2 oz. in shell)	11.9	9.3	...	613
Milk (whole)	3.3	4	5	322
Cream (average)	2.5	18.5	4.5	908
Butter	10.	8.3	...	3510
Cheese	25.2	33.7	2.4	1950
Bread (average)	9.2	1.3	53.1	1215
Rice	7.4	0.4	79.2	1620
Legumes (dried average)	24	1.7-2.3	54	1500
Potatoes	1.8	0.1	14.7	310
Green vegetables	1.4	0.2	4.8	145
Fruit (average)	0.4	0.5	8.	180

(McKillop)

Having decided how many calories a day is required according to the principles laid down on page 747, proceed to weigh out each of the articles of food that it has been customary for the person or persons to take. If the calories do not correspond to the required number, add or subtract a proper amount of one or more foodstuffs, using the figures in the fourth column of the table for this purpose. Having adjusted the food allowance according to calories, proceed to see whether there is sufficient protein according to column 1 and the principles explained on page 756. The simplest way to do this is, first, to multiply the number of ounces of each foodstuff used by 28.4 (grams to an ounce), and then by the percentage figure given in the first column. The product divided by 100 gives the grams of protein. Finally calculate by the same method the grams of carbohydrate and fat, and see that they bear the ratio to each other of about 4 to 1.

Finally, it should be remembered that the above requirements refer to foodstuffs actually digested. In the case of protein, 10 per cent is usually subtracted from the crude protein in arriving at this figure. For fats and carbohydrates the figure is quite variable. The figures in the above table are also for raw materials. Where there is evident loss in cooking, proper allowance must, of course, be made.

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CHEESE POISONING A TOXICOGENIC BACILLUS ISOLATED FROM CHEESE*

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HISTORICAL INTRODUCTION.

CHEESE poisoning, according to Husemann,[1] was already known in North Germany in the sixteenth century. Christison,[2] in his "Treatise on Poisons," stated: "During the third quarter of the last century (eighteenth) this kind of poisoning was so common that several of the German states investigated the subject, and legislative enactments were passed in consequence. For a long time the prevalent belief was that the cheese acquired an impregnation from copper vessels used in the dairies, and accordingly the Austrian, Wirtemberg, and Ratesberg states prohibited the use of copper for such purposes. This opinion was proved by chemical analysis to be untenable." Morgagni[3] mentions arsenic as the cause of cheese poisoning. Later investigators ascribed the poisonous properties to lead, zinc, and mercury.

Early in the nineteenth century the experimental method of inquiry was begun; and although it was not until the latter half of the century that the cause was definitely established, the work done in the interim was of value from the negative point of view. Hunnefeld,[4] in 1827, analyzed poisonous cheese and experimented with extracts upon the lower animals. He considered the active poisoning agents to be sebacic and caseic acids. "About the same time, Sertürner, making analyses of poisonous cheese for Westrumb, also traced the poisonous principles, as he supposed, to these fatty acids."[5] This view was adhered to by succeeding investigators until 1852, when Schlossberger proved conclusively that the fatty acids, in a pure state, were devoid of poisonous properties.

In the years 1883 and 1884 there were reported to the Michigan State Board of Health about three hundred cases of cheese poisoning. Vaughan,[6] working on the cheese sent to him for analysis, was able to isolate a ptomaïne, called by him tyrotoxicon, which he considered the active agent in the poisonous cheese. His work was corroborated by Wallace[7] in 1887, by Wesener and Rossman[8] in 1898, and by Newman[9] in 1902. Lepierre,[10] in 1894, isolated a base having the formula $C_{16}H_{24}N_2O_4$ from poisonous cheese. Dokkum,[11] in 1895, isolated a base, a currare-like poison, from putrefactive cheese, which he called tyroxin. Recently (1910) Spica[12] obtained a toxic extract, of unknown chemical composition, from poisonous cheese.

From the work of these men it is evident that they considered cheese poisoning an intoxication. In classifying the various bases obtained from poisonous cheese with the ptomaïnes, they moreover admitted the poisoning to be primarily due to bacterial activity. For a ptomaïne, according to Vaughan and Novy,[13] "may be defined as an organic chemical compound, basic in character, and formed by the action of bacteria on nitrogenous matter." The question

*A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Public Health in the University of Michigan.

then arose: "Can pathogenic or toxicogenic bacteria be shown to be present in poisonous cheese?"

Bacterial analysis of poisonous cheese had already been undertaken by Sternberg, at the request of the Michigan State Board of Health in 1884. In his report[14] to that body he stated that he found "micrococci in the fluid of the cavities of the cheese." Subcutaneous injections of the pure cultures into rabbits were without effect. He concluded: "It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above mentioned micrococcus, or of some other microorganism which had preceded it, and had perhaps been killed by its own poisonous products."

Novy,[15] in 1890, isolated an organism from poisonous cheese which, when inoculated in milk and kept in the incubator, was fatal to cats. Vaughan and Perkins,[16] in 1895, isolated a toxicogenic bacillus from cheese which had proved poisonous to twelve persons. Their bacillus belonged to the colon group. Holst,[17] a year later, described a colon-like bacillus which he had obtained from poisonous cheese. Fonteyne,[18] in 1908, obtained a Gram-positive, pathogenic bacillus from a sample of cheese which had caused a gastrointestinal epidemic at Meirelbeke among forty persons. Kühl,[19] in 1913, working with a cheese that had caused poisoning to about fourteen persons, isolated a pathogenic organism which he considered similar to the Escherich lactis aerogenes bacillus. In all these cases it was demonstrated by animal experimentation that the organism isolated caused the poisoning. Vaughan and Perkins were able to show that their organism formed a soluble poison, as sterile filtrates of their cultures, when injected intraperitoneally, into guinea pigs, caused death. In the other cases of cheese poisoning from bacterial activity no studies were made, or at least reported, upon the toxicogenic properties of the organism.

An outbreak of cheese poisoning among several members of a family in Kalamazoo, Michigan, offered the opportunity to investigate and study the cause of the poisoning. The results of this investigation are embodied in this report.

HISTORY OF THE CASES.

Mr. and Mrs. B. W. M. and family of four children, of Kalamazoo, Michigan, on June 20, 1916, were taken seriously ill several hours after having eaten supper, which had included American cheese. Dr. W. S. Tomkinson, who was called in, sent a sample of the cheese, of about 100 grams, and a history of the case to the Hygienic Laboratory of the University of Michigan. His report follows:

"Case 1. Mr. B. W. M., osteopath, ate supper, including limburger cheese, about 6 P.M. At 12 P.M. purging; vomiting; face pale, muscles trembly; eyes dilated; pulse 110, irregular. Vomitus thin, reddish color with small chunks of cheese and undigested meat. 12:10 A.M. gave tablespoonful magnesium sulphate in ℥ ii hot water. Purging profuse. O. K. at 3 A.M. with painful abdomen and hypothorax.

"Case 2. Mrs. B. W. M. Same as Case 1. Pulse 100, irregular; weak. 12 P.M., vomiting and straining, with flushed face. No purging. 12:10 gave magnesium sulphate tablespoonful to ℥ ii hot water. Vomited immediately. Repeated dose; vomited in five minutes. Patient grew worse and rolled on floor with stomach cramps. Gave ℥ i epsom salts to pint of hot water with immediate results and relief. O. K. 3 A.M.

"Case 3. Oldest boy, age 11 years. Was feeling better when I reached house at 12

midnight after purging and vomiting. Epsom salts cleaned out bowels thoroughly and gave relief.

"Case 4. Girl, age 8 years. At 6 P.M. ate heartily of cheese. 12 P.M., vomiting. Mother gave 2 ℥ castor oil. Girl quieted down and went to sleep. At 12:20 I woke her up and found she had a chill. Cold sweat. Pulse slow, irregular. She was weak and trembly. Eyes dilated, face pale, cyanotic. Said she felt all right, but was cold and wanted to be covered up and go to sleep again. Gave ℥ ss magnesium sulphate which was thrown out of stomach with such force it struck opposite wall three feet away. Many small chunks of cheese in vomitus. Washed out stomach with warm water which returned tinged red. Repeated epsom salts ℥ ss, water ℥ ii, which started purging with relief. Patient O. K. and asleep at 2 A.M. Pulse weak, rapid, but regular. Color returned to face.

"Case 5. Boy, age 6 years. Asleep at midnight was awakened and took epsom salts ℥ ss, hot water ℥ ii, and immediately fell asleep again. Slept 20 minutes, after which purged out thoroughly. Pulse 60, slow, very irregular. Pupils dilated, face cyanotic, cold sweat. Not in as much pain as rest. After purging thoroughly—face flushed—went back to bed feeling comfortable. O. K. 3 A.M.

"Case 6. Baby boy, age 16 months. Ate sparingly. Gave magnesium sulphate ℥ ss, hot water ℥ ii, and put to bed. Slight cramps; free bowel movement. No vomiting. O. K. 3 A.M.

SYMPTOMS.

The symptoms as given by Tomkinson are those of known cases of cheese poisoning,—a fact that led him to regard his cases as those of tyrotoxismus. As a rule, the symptoms are of a severer character. Early observers[20, 21, 22] gave these symptoms as characteristic of cheese poisoning: chills, paleness of face, severe vomiting, diarrhea, marked pains in stomach and intestines, trembly limbs, thirst and dizziness. Pollius,[23] Wallace,[7] and Holst[17] mention vomiting of blood. Fonteyne[18] stated that some of his patients passed bloody stools. At times marked nervous disturbances may occur, as in a case cited by Vaughan,[16] where a patient became violently delirious. In the Michigan epidemic of 1883-1884[24] the symptoms produced by poisonous cheese were: dryness of the throat, nausea, vomiting, diarrhea, nervous prostration, headache, and sometimes double vision.

INCUBATION PERIOD.

Significant and important is the interval of time between the eating of the cheese and the appearance of the first symptoms of poisoning,—an interval which must be regarded, from the majority of cases, as an incubation period. This terminology would imply that cheese poisoning should be regarded not as an intoxication but rather as an infection. This is, indeed, the opinion of the present-day investigators.

The incubation period in the cases with which this report is concerned was, it may be remembered, six hours. It would be reasonable to assume that, were we dealing with an intoxication, the symptoms would have appeared much earlier. Instances are cited in the literature[1, 20, 21] where the interval of time between the eating and the appearance of the symptoms was from one to two hours. Westrumb[2] mentioned half an hour interval. In the Michigan epidemic of 1883-1884 some persons were taken ill from thirty minutes to four hours after eating. In such cases a preformed poison may be considered, like that of Vaughan's tyrotoxicon. In all cases, however, where bacteria were isolated

from poisonous cheese, the interval was not less than five or six hours,—a period amply sufficient for bacterial activity.

DURATION OF SICKNESS.

Prompt treatment by the attending physician resulted in a rapid recovery by the Kalamazoo patients. The poisoning, however, to which these people were subjected, was not so marked as in many of the cases cited in the literature. To this can be attributed the short duration of their sickness. Instances are not lacking where days and even weeks elapsed before the recovery of the patients. Thus Grimm[20] stated the period to be three days; Pollius,[23] eight to twenty-four hours; Zenker,[22] twenty-four hours; Wallace,[7] three days; Ehrhardt,[25] "a long convalescence;" Newman,[9] forty-eight hours; Hughes and Healey,[26] four to eight days; Holst,[17] four days to three weeks; and Kühl,[19] ten days.

MORTALITY.

Deaths through cheese poisoning make up a very small percentage of the total number of cases. In the Michigan epidemic no fatalities were recorded. Lochte[27] tabulated eleven fatal cases from cheese poisoning up to 1903. Berg[28] mentions a fatal case occurring in 1908.

POSTMORTEM APPEARANCE.

Not all the fatal cases gave the same postmortem pictures. Hunnemann[1] found the stomach and intestines to be inflamed. Cameron,[29] quoting Holm's[30] postmortem findings, said "There was extravasated blood in the lungs, which were slightly congested; they decrepitated. The left ventricle of the heart contained liquid blood; the right auricle and two left cavities contained numerous soft and recent clots. The stomach and other abdominal viscera did not present any abnormal appearances which would account for death." Hughes and Healey,[26] in their report of an epidemic of gastroenteritis attributed to cheese poisoning (tyrotoxicon having been found in the cheese) at Aldershot, England, in 1899, gave the following postmortem appearances in three fatal cases: "Intense congestion; inflammation and edema of the mucous membrane of the stomach and small intestines, varying in degree and locality, but in one case involving the stomach and the whole of the small intestine. Peyer's patches were practically unaffected, but the solitary glands were congested and prominent. There was no ulceration or visible entozoa and the colon was unaffected. In one case the small intestine contained loose terra-cotta colored fecal material. The liver, which was enlarged (from 70 to 78 ounces) and bile-stained, showed signs of fatty degeneration. The gall bladder was full but not markedly distended. The spleen was normal. The kidneys were enlarged and intensely congested; the capsule was nonadherent. In one case the cortex of both kidneys contained small cysts filled with clear fluid. The skin and other tissues were deeply bile-stained. The lungs were congested posteriorly. The thoracic and abdominal lymphatics were not noticeably enlarged." Berg's case[28] presented a picture of cholera nostras. It seems, however, that in many cases marked anatomical changes do not occur.

APPEARANCE OF THE CHEESE.

Tomkinson, the physician attending the M. family, sent a sample of the cheese and also of the meat eaten at that meal. The meat, evidently a pork chop, was found on bacterial examination, to contain only Bacillus mesentericus vulgatus, and hence was dismissed from consideration as the source of poisoning. The sample of cheese was of about 100 grams in weight, of the variety known as American (wrongly termed Limburger by Tomkinson), and of normal appearance and odor. This normal appearance corroborates the statements of many investigators that the cheeses causing poisoning were seemingly in excellent condition.

EXPERIMENTAL WORK.

Feeding Experiments.—Experiments were conducted with a view of determining whether poisoning could be induced in laboratory animals by feeding them the suspected cheese.

TABLE I.

FEEDING SUSPECTED CHEESE.

June 24, 1916.	Guinea pig No. 1, 220 gm., fed 10 gm. cheese.	No effects.
June 24, 1916.	Rats No. 1 and No. 2, both fed 5 gm. cheese.	No effects.
June 24, 1916.	Mice No. 1 and No. 2, both fed 5 gm. cheese.	No effects.

None of the animals fed showed the least symptoms of sickness. This was to be expected in view of similar findings by other authors. Nückel,[31] in 1836, stated that a dog was unaffected eating cheese that had caused marked poisoning in a man. Pollius[23] claimed to have observed trembling in a mouse fed with poisonous cheese. Holm[30] obtained negative results in his feeding experiments. Sternberg[14] reported negative results by feeding rats and dogs. Vaughan said,[32] "If two samples, one of good, the other of poisonous cheese, were placed before a dog or cat, the animal would invariably select the good cheese; but if only poisonous cheese was offered, and the animal was hungry, it would partake freely. A cat was kept seven days and furnished only poisonous cheese and water. It ate freely of the cheese and manifested no untoward symptoms. After the seven days the animal was etherized and abdominal section was made. Nothing abnormal could be found." Holst[17] and Kühl[19] also obtained negative results in their feeding experiments.

ALCOHOLIC EXTRACTS OF CHEESE.

After removing aseptically a portion of the suspected cheese for bacterial analysis the remainder, 65 grams, was chopped up finely and digested with 150 c.c. absolute alcohol for three days at room temperature. It was then filtered, the filtrate evaporated to dryness, taken up in 50 c.c. distilled water, heated on a water bath, and filtered into a weighed dish. On evaporation to dryness a residue of 1.634 gm. was obtained. This was then taken up in 50 c.c. distilled water, and portions of 5 and 10 c.c. each were injected, intravenously, into guinea pigs. The animals showed no symptoms of disturbance. The same procedure with the sample of the pork chop also gave negative results. It was concluded, therefore, that no alcohol-soluble substance caused the poisoning, and that the source might possibly be traced if a bacterial analysis were made.

ISOLATION OF THE ORGANISM—TECHNIC.

With a sterilized knife a section of cheese was cut so that all surfaces were free from external contamination. This was then placed in a sterile test glass, physiological salt solution added, the cheese thoroughly macerated with a sterile rod, and the resulting suspension filtered through a sterile cotton filter. 1 c.c. portions of the filtered suspension were then inoculated into glucose gelatin, milk, and bouillon tubes, and the latter two incubated at 37° C. for 24 hours. A layer of sterile oil to about a quarter of an inch in depth was put over the glucose gelatin and these tubes incubated at room temperature. At the end of 24 hours the glucose gelatin showed marked gas formation, the milk was curdled, and the bouillon had a very heavy growth.

From these three different media, after plating on agar and fishing numerous colonies, an organism was isolated which, when grown in milk, proved pathogenic to mice. The same organism was isolated from glucose agar plates, grown anaerobically in Novy jars, in an atmosphere of hydrogen gas.

MORPHOLOGY.

The organism is a facultative anaerobic bacillus, resembling in form and dimensions the Bacillus colon. The size of the bacillus varies from coccus-like forms to large rods, depending upon the kind and age of the media in which it is grown. As a rule its length is about three times its width. The organism grows singly and also in the diplo form. Thread formation has been infrequently observed. It does not form spores.

MOTILITY.

Depending upon the media and the conditions of growth the bacillus may show active motility, sluggish motility, or even marked Brownian movement. Table II shows the influence of the kind of media upon the size and motility of the organism.

TABLE II.

THE INFLUENCE OF MEDIA UPON SIZE AND MOTILITY.

AGE	MEDIUM	SIZE	MOTILITY
24 hours	Bouillon	Small rods; several large rods	Actively motile, large rods sluggishly motile
24 hours	Agar	Small rods	Actively motile
72 hours	Agar	Coccus-like bacilli	Motile
24 hours	Lactose broth	Slender rods, many large	Sluggishly motile, a few actively
24 hours	Dextrose broth	Plump rods, some very long	Marked Brownian movement; few slightly motile
24 hours	Saccharose broth	Small rods	Actively motile; many show no motility
24 hours	Milk	Small rods	Actively motile
72 hours	Milk	Small rods	Motile
24 hours	Uschinsky's medium	Fairly long rods	Many actively, some sluggishly motile
24 hours	Bile lactose	Small slender rods	Marked Brownian movement
Peritoneal fluid from guinea pig		Small plump rods	Few actively motile; most marked Brownian movement

STAINING PROPERTIES.

The organism takes all stains readily. With Loeffler's methylene blue the ends of the bacillus stain a dark blue, while the inner portion resembles a vacuole. It is Gram-negative and not acid-fast. Smears made from the organs of an animal killed by the bacillus, when stained according to the Welch method, show the organisms to have capsule-like bodies around them. These, however, could never be verified as capsules.

GROWTH ON GELATIN.

On gelatin plates the colonies are usually of two forms,—oval, characteristic of the deep colonies, and round, with irregular borders, characteristic of the surface colonies. Stabs show filiform growths, the growth being best at top. The gelatin is not liquefied.

GROWTH ON AGAR.

The surface colonies on agar plates are round, with regular borders. Distinct concentric rings from two to three in number can be observed,—the inner ring being darker. The deep colonies are oval. When grown on glucose agar plates, large bubbles of gas, at times approaching 1.5 cm. in diameter, are formed. On agar slants the growth is abundant, filiform, and glistening. An odor is given off strongly resembling that of B. coli cultures. No pigment formation has been observed. On Drigalski-Conradi medium it forms pink colonies similar to B. colon. On Endo medium the colonies are red with the characteristic metallic caps of colon colonies.

GROWTH ON POTATO.

The growth on potato is noncharacteristic. Depending upon the reaction of the potato the growth may be scanty or abundant—a slightly alkaline reaction favoring the latter. Where abundant, it is of a slimy consistency, raised, and at times spreading. After several days the potato becomes more or less browned.

GROWTH IN NUTRIENT BROTH.

The bacillus grows readily and rapidly in nutrient broth, distinct clouding being visible in as short a period as three hours. A slight ring forms at the surface, but this may, at times, be lacking. No pellicle formation takes place. The clouding is strong at the end of twenty-four hours. On standing several days a flocculent sediment forms, with a slight clarification of the culture. The odor here again resembles that of a B. colon culture. In sugar broths growth is abundant, with formation of gas and acids.

GROWTH IN MILK.

When first isolated the bacillus would curdle milk at the end of about four to five days. The curd in that case would be slimy. After passage through many animals (approximately 80), the organism curdled milk in forty-eight hours, and the curd was distinct from the whey. Gas bubbles could be observed at the surface of the culture. The acidity formed varied from 0.2 per cent to 0.3 per cent.

GROWTH ON BLOOD SERUM.

On this medium the organism forms a moderate, slightly glistening, filiform growth.

GROWTH IN USCHINSKY'S SOLUTION.

The solution was similar to that used by Vaughan and Perkins and was made up as follows:

Glycerine	40 parts
NaCl	1 part
$CaCl_2$	0.1 part
$MgSO_4$	0.4 part
K_2HPO_4	2.5 parts
Ammonium Lactate	6.0 parts
Asparagin	3.4 parts
Water	1000 parts

The bacillus grew copiously in this solution. In Fränkel's modification of Uschinsky's medium no growth was observed at twenty-four hours. A light growth was visible at forty-eight hours, which increased to a heavy growth at seventy-two hours. The laboratory strain of B. colon, and a strain isolated from feces also showed no visible growth in this solution at twenty-four hours, while both gave good growths in the Uschinsky medium for the same period. This is interesting in view of the fact that Vaughan and Perkins found their organism would not grow in Uschinsky's medium if the dipotassium hydrogen phosphate were replaced by disodium hydrogen phosphate. The latter salt, as can be seen from the following formula, was used in Fränkel's medium:

Asparagin	4.0 gm.
Ammonium Lactate	6.0 gm.
Na_2HPO_4	2.0 gm.
NaCl	5.0 gm.
Water	1000 c.c.

TEMPERATURE REQUIREMENTS.

The optimum temperature is about 37° C. The organism grows also well at room temperature. At high temperatures, as at 45° to 50° it seems to lose its power of fermenting sugars. It grows scantily below 10° C. Cultures grown at 37° C. appear to be the most toxic.

THERMAL DEATH POINT.

The thermal death point of the organism was determined by both the moist and dry heat methods, using silk thread, according to the technic employed in the Hygienic Laboratory of the University. The bacillus, as can be seen from the following tables, although withstanding moist heat at 50° C. for two hours is readily killed at 60° C. It is practically nonresistant to dry heat.

TABLE III.

THERMAL DEATH POINT. MOIST HEAT.

Time in Minutes	½	1	2	3	5	10	15	30	45	60	120
50° C.							+	+	+	+	+
55° C.							+	−	−	−	−
60° C.		+		−	−	−	−	−			
70° C.		−		−	−	−	−				
100° C.	−	−	−	−	−						

TABLE IV.

THERMAL DEATH POINT. DRY HEAT.

Time in Minutes	5	15	30	45	60
100° C.	+	–	–	–	–
120° C.	–	–	–		
130° C.	–	–	–		
150° C.	–	–	–		

Controls for both moist and dry heat = +.

FERMENTATION.

Various sugars were used to test the fermenting property of the organism and also to differentiate it thereby, if possible, from the colon bacillus. The media used were 2 per cent sugar broths neutral to phenolphthalein. Before inoculation the tubes were allowed to stand at room temperature for 12 hours. Two colon strains were used as comparatives: one, the laboratory strain, and the other, a strain isolated from feces. Loopfuls from 24 hours' cultures of each of the three organisms were inoculated into the fermentation tubes, and these incubated at 37° C. Readings were taken at intervals of 24, 48, and 72 hours.

For convenience the bacillus isolated from the cheese will be termed Bacillus 3B.

TABLE V.

FERMENTATION COMPARATIVES. PER CENT GAS.

	B. COLON FECES			B. COLON LAB.			BACILLUS 3B.		
Hours	24	48	72	24	48	72	24	48	72
Xylose	10	25	30	10	25	30	20	30	30
Arabinose	15	25	30	15	30	30	15	25	25
Dextrose	30	30	30	15	15	20	25	25	25
Mannose	15	15	20	20	20	25	15	15	20
Galactose	15	15	25	15	20	20	25	25	25
Mannite	50	70	70	15	30	30	40	60	60
Dulcite	–	–	10	–	30	50	5	30	40
Lactose	30	40	40	30	40	40	30	40	40
Maltose	10	30	30	15	40	40	15	30	30
Saccharose	–	–	–	5	40	40	20	25	30
Raffinose	–	–	–	15	50	60	10	25	30
Inulin	–	–	5	–	5	5	–	–	–
Dextrin	10	10	15	5	10	10	5	10	10
Starch	–	–	–	–	5	5	–	–	–
Glycogen	–	–	–	–	–	–	–	–	–

It is evident that the fermentation method can not be used as a means of distinction between the B.colon and bacillus isolated from the cheese. The latter readily ferments the pentoses, hexoses, di- and trisaccharides. Of the polysaccharides it ferments only dextrin. It was thought, for a time, that its nonfermenting of inulin might be of help in distinguishing it from B. colon. Further comparative tests, however, showed that this was not feasible, since the laboratory and feces strains did not always ferment it, and that at times a bubble or two of gas would be formed in tubes inoculated with the bacillus from the cheese.

With the exception of Vaughan and Perkins other investigators did not attempt to study the fermenting properties of their organisms to any great extent. Holst[17] and Kühl[19] merely report that their organisms fermented lactose. Fonteyne[18] found both dextrose and lactose to be nonfermented. Vaughan and Perkins[16] showed that their bacillus fermented dextrose, lactose, saccharose, maltose, dextrin, starch, and glycogen.

NITRATE REDUCTION AND INDOL FORMATION.

The bacillus reduces nitrates to nitrites, but to a smaller degree than the colon bacillus. Using the same strength of nitrate solution the reduction for the Bacillus 3B is two-fifths that for B. colon. It also forms indol.

PATHOGENICITY.

When first isolated the bacillus proved to be pathogenic to mice only. Injections of the original cheese suspension into guinea pigs and rats had no effects. One c.c. of a pure culture of the organism grown in milk killed a mouse, when injected intraperitoneally, in 8½ hours. The organism was recovered from the heart blood, grown in milk, and again injected into a mouse. The mouse died within 10 hours. A rat injected with the same culture (1 c.c.) showed symptoms of being unwell, but did not die. After passage through three more mice the organism had increased in virulence to kill a rat. A guinea pig inoculated with the same culture that killed the rat was unaffected.

The organism was then passed through four rats, and after the last passage was isolated from the heart blood, plated on agar, and obtained in pure culture. It was then grown in milk for 24 hours at 37° C. and 1 c.c. injected, intraperitoneally, into a guinea pig. Death occurred in about 20 hours. The bacillus was then passed through two successive guinea pigs and again isolated in pure culture. Owing to the rush of other work the agar slant was left in the incubator (37° C.) for about 25 days; and at the end of that time it was found that the organism had lost its fermenting power and also its pathogenicity.

It was thought that possibly, owing to its selective media, the bacillus would gain in virulence by repeated transplanting into milk or lactose broth. Such was, indeed, the case. A tube of lactose broth was inoculated with the organism from the stock culture, incubated at 37° C. for 24 hours, and then transplanted into lactose broth. After five successive transplants the organism was found to be again pathogenic to guinea pigs.

That the lactose increased the virulence of the organism seemed evident. Dextrose had a similar effect. A tube each of bouillon, lactose bouillon, milk, and an agar slant were inoculated with the bacillus, incubated at 37° C. for 24 hours, and then 1 c.c. from each of the cultures injected, intraperitoneally, into guinea pigs, the agar slant being taken up in 10 c.c. of physiological salt solution. The pigs injected with the lactose bouillon and milk died in 8 hours; that with the bouillon culture, in 20 hours; while the pig injected with the suspension from the agar slant did not die until 28 days later. The explanation undoubtedly lies in the fact that an anaerobic condition, produced by the presence of sugars, increases the pathogenicity of the organism.

Occasionally some animals seemed to be refractory to seemingly fatal doses. Rats, especially, would not always show the same susceptibility. In order of susceptibility can be placed mice, guinea pigs, rabbits, cats, rats, and dogs.

The bacillus was passed through twenty guinea pigs and then isolated from the heart blood of the last pig. It was found that the virulence of the organism had increased so that .25 c.c. of a 24 hour lactose culture would kill a 200 gm. guinea pig within 20 hours. Intravenous injections did not seem to indicate any greater virulence than intraperitoneal. Subcutaneous injections were also fatal.

POSTMORTEM APPEARANCE OF GUINEA PIGS.

The picture presented by a guinea pig killed with a fatal dose of the organism was usually that of a marked peritonitis. As a rule the lungs were slightly distended, the heart in diastole, and filled with blood. The liver, kidneys, and spleen were normal, although the latter at times was dark and slightly enlarged. The amount of peritoneal fluid present was variable, ranging from none to 5 or even 6 c.c. Usually it was present in an excess from the normal. This fluid was highly toxic, and killed cats which were not killed by lactose broth cultures of the organism. In most guinea pigs there was marked inflammation at the point of injection.

DISTRIBUTION OF THE ORGANISM.

The bacillus is found in the heart blood, lungs, kidneys, liver, spleen, bile, and the peritoneal fluid. In greatest numbers, and in practically pure culture it is found in the peritoneal fluid. In the liver and heart blood it is present in small number. It is numerous in the spleen, kidneys, lungs and bile.

EFFECTS ON CATS.

On September 29, 1916, a cat which had been starved for 24 hours was injected, I.P., with 4.5 c.c. of a 24 hours' lactose broth culture. The cat soon showed signs of unrest, and attempted to vomit. She remained in a comatose condition with high fever, the temperature reaching 41.6° C. 35 hours after injection. She recovered on the second day. The temperature of normal cats was then established previous to a series of tests as to their febrile reactions with the organism. Four cats were used, the temperature taken once daily for four days.

TABLE VI.

NORMAL TEMPERATURE OF CATS.

DATE	TIME	CAT 1	CAT 2	CAT 3	CAT 4
Oct. 4	10.00 A.M.	38.05	38.15	38.65	38.10
5	11.30 A.M.	38.45	37.80	38.40	38.80
6	2.45 P.M.	39.10	38.40	38.80	39.50*
7	10.30 A.M.	38.30	38.45	39.00	37.90
Average Temperature		38.47	38.20	38.71	38.26
Average Temperature of the four cats.....					38.41

*Not included in the average, as the cat was highly excited.

Several cats were then subjected to injections of the organism and their temperatures taken.

October 9. Cat 4. Weight 3250 gm. This cat was injected, I.P., with 5 c.c. of a 24 hour lactose broth culture, at 9:45 A.M. Retching, vomiting, and purging took place within half an hour.

Temp. at	9.45 A.M. 38.20	Temp. at	2.30 P.M. 41.18
	10.45 " 39.62		4.30 " 40.50
	11.45 " 40.12	Oct. 10,	9.30 A.M. 39.10
	1.40 P.M. 40.58		12.00 " 38.50

The cat recovered.

October 10. Kitten 2. Weight 580 gm., was given, I.P., 3 c.c. of a 48 hour culture (lactose broth) at 9.45 a. m. Retching, vomiting, and purging took place in 35 minutes.

Temp. at	9.45 A.M. 37.12	Temp. at	3.15 P.M. 39.16
	10.45 " 38.76		4.15 " 39.20
	11.45 " 40.15		5.15 " 39.50
	1.15 P.M. 39.78		8.00 " 38.86
	2.15 " 39.30		10.00 " 38.44

The kitten recovered.

October 11. Cat 2, weight 2500 gm., was given I.P., 8 c.c. of an 18 hour milk culture of the organism at 9.15 A.M. Retching, vomiting, and purging took place in 30 minutes.

Temp. at	9.15 A.M.	38.12	Oct. 12, at	10.00 A.M.	39.88
	10.15 "	38.80		11.15 "	40.10
	11.15 "	38.42		1.30 P.M.	39.58
	1.00 P.M.	38.72		3.30 "	39.10
	3.00 "	38.74		4.45 "	39.62
	8.00 "	40.00	Oct. 13 at	9.45 A.M.	38.04
	10.00 "	39.40			

The cat refused food for two days. It is interesting to note that with the milk culture a longer sustained fever was obtained than with the lactose broth cultures.

Allusion has already been made to the marked toxic effect of the peritoneal fluid of animals killed by the bacillus. This fluid was of a much higher potency than either the milk or lactose broth cultures.

November 25. Cat 1, which had recovered from a dose of the pure culture given on September 29, was injected, I.P., with 5 c.c. of peritoneal fluid from Rabbit 116, killed by a lactose broth culture of the organism. Retching, vomiting, and purging took place in 20 minutes. The animal was found dead on the morning of the 26th. At the autopsy the heart was found to be in diastole, the blood being fluid. The lungs were slightly distended; the kidneys, hyperemic; the spleen, normal; and the liver, somewhat pale. No fluid was found in the peritoneum, although the viscera were moister than normally. The intestines were pale. The bacillus was recovered from the heart-blood and from the bile, and was shown to be present in smears made from the liver, lungs, spleen, and kidneys.

November 25. Cat 4. This cat was injected, I.P., with 2.5 c.c. of peritoneal fluid from a guinea pig killed by the bacillus. Retching, vomiting, and purging followed within half an hour, the vomiting and purging continuing, intermittently, for about 8 hours. The cat was found dead on the morning of the 27th. The autopsy showed the same picture found in Cat 1. The organism was recovered from the heart blood and bile, and was again shown to be present in the smears made from the liver, lungs, spleen, and kidneys.

December 1. Cat 2 was given, I.P., 3 c.c. of peritoneal fluid from a rabbit killed by the bacillus 3B. Retching, vomiting, and purging took place in 35 minutes.

Temp. at time of injection,	10.00 A.M.	37.00
	11.00 "	37.60
	12.00 "	37.48
	1.15 P.M.	40.20
	2.15 "	39.28
	3.15 "	39.56
	4.15 "	39.20
	5.15 "	40.40
Dec. 2,	9.30 A.M.	38.48

The cat died at 5 P.M. on the 2nd. The postmortem findings were similar to those of the preceding cats. The bacillus was shown to be present in the same organs.

December 8. Kitten 1, weight 650 gm., was given, I.P., 2 c.c. of peritoneal fluid from a guinea pig. Retching, vomiting, and purging took place in half an hour.

Temp. at time of injection,	9.40 A.M.	37.32
	10.40 "	39.00
	11.40 "	39.00
	1.30 P.M.	39.90
	2.30 "	39.56
	3.30 "	39.50
	4.30 "	38.80
	5.30 "	38.70
	9.00 "	35.80

The kitten died late in the night. The postmortem findings were similar to those already described.

It is interesting to note that the temperature rose higher in the cats than in the kittens, although both were equally susceptible to the organism.

To summarize the tests with cats: The symptoms produced were retching, vomiting, and purging, generally within half an hour after injection. The stools were watery and copious. Extreme weakness so that the animal could not voluntarily stand up, and inability to eat food even after a previous starvation of 24 hours were characteristic. A marked fever was produced, the rise in temperature being marked the first few hours after injection. In all cases where the cats were injected with the peritoneal fluid death occurred; where given the lactose broth or milk cultures they recovered. Half-grown cats seemed to be the best adaptable for experimental purposes.

EFFECTS ON RABBITS.

Rabbits proved also susceptible to the organism. As with the cats the peritoneal fluid from animals killed by the bacillus was highly toxic to rabbits. The temperature did not reach the maximum obtained in the cats, although it showed an appreciable rise. In many of the rabbits there was a marked collapse, at times approaching paralysis. As a rule, large doses of the culture were necessary to cause death.

November 24. Rabbit 114 was injected, I.P., at 10.15 A.M., with 4 c.c. of peritoneal fluid from a guinea pig. Death occurred in 4 hours. The heart was in diastole; the blood, fluid. The blood vessels were distended. The organs appeared normal. There was very little fluid in the peritoneum.

November 24. Rabbit 116 was injected, I.P., at 9 P.M., with 4 c.c. of peritoneal fluid from a guinea pig. The rabbit was found dead early the next morning. The autopsy gave the same findings as those of rabbit 114, with the exception that there were about 5 c.c. of a dark fluid in the peritoneum. The bacillus was demonstrated in smears made from the lungs, kidneys, spleen, liver, heart blood, bile, and the peritoneal fluid.

December 1. Rabbit 133 was injected, I.P., with 1.5 c.c. of peritoneal fluid from a guinea pig. Temperature readings were taken at regular intervals.

Temp. at time of injection,	10.00 A.M.	38.35
	11.00 "	37.98
	12.00 "	38.38
	1.15 P.M.	38.72
	2.15 "	38.84
	3.15 "	39.82
	4.15 "	39.92
	5.15 "	39.40
Dec. 2,	9.30 A.M.	38.40

The rabbit died at 3.00 P.M. on December 2. The post mortem findings were similar to those already described.

Intravenous injections proved fatal when 1.5 c.c. of a 24 hour culture (lactose broth) were given.

December 8. Rabbit 160 was injected, I.V., with 1.5 c.c. of a 24 hour lactose broth culture at 10.30 A.M. The rabbit died early in the morning on the following day.

December 8. Rabbit 170 was injected, I.V., with 1.0 c.c. of a 24 hour culture (lactose broth) at 9.30 A.M.

Temp. at	9.30 A.M.	38.35
	10.30 "	39.30
	11.30 "	39.56
	2.00 P.M.	39.96
	3.00 "	39.48
	4.00 "	38.64
	5.00 "	38.90

The rabbit recovered.

EFFECTS ON DOGS.

The effect of feeding dogs with the pure culture of the organism was tried. Finely chopped meat was saturated with a lactose broth culture of the bacillus and given to two dogs, who ate it with apparent relish. No ill-effects were visible.

December 13. A small dog, 3.5 Kg., was given, I.P., at 2 P.M., 5 c.c. of a 24 hour lactose broth culture of the bacillus. No retching nor vomiting, but purging was very marked. The temperature range for the period immediately after injection was as follows:

2.00	P.M.	38.3
3.00	"	39.5
4.30	"	40.1
5.30	"	40.1
7.00	"	39.2
8.00	"	38.8

The dog recovered slightly, but refused food or ate very sparingly. Death occurred early on the morning of December 19. Autopsy: The blood vessels of the mesentery and peritoneum were enlarged. The heart was enlarged and the blood fluid. The vena cava was distended; the veins throughout the body were congested. The gall bladder was distended; the liver was large, with marked hyperstatic congestion. The lungs were normal. The kidneys were enlarged and hyperemic; when cut, they showed many bleeding points. The periphery was of dark color. The bacillus was recovered from the heart blood.

EXPERIMENTS WITH MILK CULTURES.

With the view of determining whether tyrotoxicon was formed by the bacillus, experiments were conducted with milk cultures. The method of procedure was as follows:

Skimmed milk in 1000 c.c. portions was sterilized in the autoclave at 115° C. for 20 minutes. It was then inoculated with a loopful of the organism from a 24 hour agar slant, and incubated at 32° C. to 35° C. for 30 days. At the end of the incubation period the whey was separated from the curd by filtration through paper, and made sterile by filtration through a Berkefeld candle. The whey was then made alkaline with sodium hydrate and extracted with ether.

Vaughan and Perkins found that ether from certain commercial houses, on evaporation, left a residue which, when taken up in water and injected into guinea pigs, caused death. In all the work with ether alkaline extracts, therefore, controls were run on the purity of the ether. In no case was the ether found to contain a toxic residue.

The ether extract obtained from the whey was in the form of a gel, and when evaporated to dryness, left a caramel-colored residue, soluble in water. At no time were crystals obtained, and while the extract showed a slightly poisonous action, no tests made indicated the presence of tyrotoxicon.

The curdling of the milk, as a rule, took place within seven days; but the curd was, even at the end of a month, rather viscous and difficult to separate from the whey. The whey was always strongly acid to litmus. Intraperitoneal injections of the sterile whey into guinea pigs in 5 c.c. and 10 c.c. quantities had no effects. That the organism was not killed by its metabolic products were proved by inoculating bouillon tubes with a loopful of the whey; growth was always obtained, and sugars were fermented.

The alkaline ether extract, when taken up in water, gave a neutral reaction to litmus. In one or two of the portions the reaction was faintly acid. It had usually a rancid, almost putrid odor, and a slightly irritating taste. No positive

Biuret and Millon tests were given, although in one or two of the portions there seemed to be very faint positive reactions. In all of the extracts a strongly positive α naphthol test was given.

The data in Table VII shows that the extract is slightly toxic. The alkaline ether extract was taken up in sterile water so that 10 c.c. represented the liter of the original milk.

TABLE VII.

THE EFFECTS OF ALKALINE ETHER EXTRACTS OF WHEY UPON GUINEA PIGS.

DATE	GUINEA PIG	INJECTION	AMOUNT	RESULT
Dec. 7	1	I.V.	2.0 c.c.	Death in 20 hours
7	2	I.V.	2.0 c.c.	Sick; recovered
7	3	I.V.	5 c.c.	Death in 48 hours
7	4	I.V.	7 c.c.	Death in 22 hours
8	5 to 10 inc.	I.V.	1 c.c.	No effects
8	11	I.P.	10 c.c.	Death in 7 days
12	12 to 14 inc.	I.V.	5 c.c.	Sick; all recovered

Attempts were made to obtain, if possible, the poisonous part in the extract by condensation through alternating freezing and thawing, a process used successfully in this laboratory by Ned R. Smith in his work on water and alcohol organ

TABLE VIII.

PROTECTION ACQUIRED BY GUINEA PIGS THROUGH ETHER EXTRACTS.

Oct. 27	G. P. 1 given	1 c.c. of extract, I.P. No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of B. 3B; death in 12 days
Oct. 27	" 2 "	1 c.c. of extract I.V. No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of 3B; death in 16 days
" 2	Control G. P.	1 c.c. of a 24 hour culture of 3B; death in 12 hours
Oct. 27	G. P. 3 given	2 c.c. of extract, I.V. Shock; recovered
" 30	" " "	1 c.c. of 24 hour culture of 3B; death in 14 days
" 30	Control G. P.	1 c.c. of a 24 hour culture of 3B; death in 12 hours
Dec. 8	G. P. 4 given	1 c.c. extract, I.V. No effects
" 12	" " "	0.5 c.c. extract, I.P.
" 21	" " "	1 c.c. of 24 hour culture of 3B. No effects
" 8	" 5 "	1 c.c. extract, I.V. No effects
" 12	" " "	1.25 c.c. extract, I.P.
" 21	" " "	1 c.c. 24 hour culture 3B. No effects
" 8	" 6 "	1 c.c. extract, I.V. No effects
" 12	" " "	1.5 c.c. extract, I.P.
" 21	" " "	1 c.c. 24 hour culture 3B. Death in 4 days
" 21	Control G. P.	for G. P. 4, 5, 6 given 1 c.c. of a 24 hour culture 3B. Death in 18 hours
" 12	G. P. 7 given	5 c.c. extract, I.V. No effects
" 17	" " "	5 c.c. " I.P.
" 21	" " "	5 c.c. " I.P.
" 26	" " "	1 c.c. of 24 hour culture 3B. No effects
" 12	" 8 "	5 c.c. extract, I.V. No effects
" 17	" " "	5 c.c. " I.P.
" 21	" " "	5 c.c. " I.P.
" 26	" " "	1 c.c. of 24 hour culture 3B. Death in 11 days
" 12	" 9 "	5 c.c. extract, I.P. No effects
" 17	" " "	5 c.c. extract, I.P.
" 21	" " "	" " "
" 26	" " "	1 c.c. of 24 hour culture of 3B. Death in 20 days
" 26	Control G. P.	for G. P. 7, 8, 9, given 1 c.c. of a 24 hour culture of 3B. Death in 5 hours

extracts. The alkaline ether extract was taken up in sterile water, put into a freezing mixture, allowed to freeze, thawed at room temperature, frozen again, etc., until the solution showed two distinct layers, a colored and a colorless layer. The colored layer was pipetted off, and portions injected into guinea pigs. I.V. injections of the colored portions in 1 c.c. doses had no effects; 2 c.c. killed a guinea pig in 6 days; 2 c.c. of the colorless portion, injected I.P., killed a guinea pig in 4 days. On January 25, four guinea pigs were injected, I.P., with 1 c.c. each of the colored portion. Two died in three days, while the remaining two were unaffected.

A striking feature of the experiment with the alkaline ether extracts was the protection acquired by guinea pigs against fatal doses of the germ after they had been injected with the extract. This is shown in Table VIII.

Of the nine guinea pigs inoculated with the alkaline ether extract three were unaffected by fatal doses of the organism. The remaining six died after an interval ranging from four days to twenty days. The controls succumbed promptly from five to twenty hours. From these results it is evident that a fair degree of protection can be obtained against fatal doses of the organism, a protection which may approach immunity. Milk is not a good medium for the elaboration of the poison, but may become so under favorable conditions. The bacillus does not form tyrotoxicon.

FILTRATES OF LACTOSE BROTH CULTURES.

The marked pathogenicity of the organism when grown in lactose broth and the slightly toxic products obtained in milk cultures led to the study of the toxicity of sterile filtrates from lactose broth cultures. For the first test a broth containing 2% lactose and 5% glycerine was used. The broth, neutral to phenolphthalein, was inoculated with the bacillus, and incubated at 32° C. to 35° C. for 9 days. Several drops of tricresol were then added, and the culture filtered on the following day through a Berkefeld candle. The filtrate was tested for sterility.

On December 21 three guinea pigs were inoculated with increasing amounts: 0.5 c.c., 1 c.c. and 2 c.c. of the sterile filtrate. The pigs were restless for a short time but otherwise showed no ill effects. The filtrate was allowed to stand in a test glass, in a dark place, until January 17, 1917, on account of press of other work. Considerable condensation had taken place. When inoculated in 1 c.c. amounts into guinea pigs, intraperitoneally, it now proved fatal to two out of five of these animals, death occurring in 20 hours. On January 18 six more guinea pigs were inoculated with the following results:

G.P. 1	2 c.c.	I.V.	Death in 27 hours
G.P. 2	1 c.c.	I.V.	Death in 72 hours
G.P. 3	1 c.c.	I.V.	Death in 96 hours
G.P. 4	1 c.c.	I.P.	Death in 24 hours
G.P. 5	1 c.c.	I.P.	Death in 24 hours
G.P. 6	1 c.c.	I.P.	Recovered

The heart blood of each pig was found to be sterile. Marked inflammation of the intestines was characteristic in all cases. The organs were normal.

Tests were next made to determine whether the presence of glycerine was necessary or desirable. Three different broths were inoculated with the organism, incubated at 32° to 35° C. for 7 days, several drops of tricresol added, and the cul-

tures filtered on the following day through Berkefeld candles. The filtrates were then tested for sterility.

Broth A contained 2 per cent lactose.
Broth B contained 5 per cent glycerine.
Broth C contained 5 per cent glycerine, 2 per cent lactose.

TABLE IX.

COMPARATIVE TOXICITY OF LACTOSE, GLYCERINE, AND LACTOSE-GLYCERINE FILTRATES.

DATE	G.P.	AMOUNT INOC. I.P.	FILTRATE	RESULT
Jan. 31	1	1.0 c.c.	A	Death in 4 hours
31	2	1.5 c.c.	A	Death in 7½ hours
31	3	2.0 c.c.	A	Sick; recovered
31	4	1.0 c.c.	C	Sick; recovered
31	5	1.5 c.c.	C	Death in 4 hours
31	6	2.0 c.c.	C	Death in 6 hours
Feb. 1	7	1.0 c.c.	A	Death in 18 hours
1	8	1.5 c.c.	A	Death in 40 hours
1	9	1.0 c.c.	B	Sick; recovered
1	10	1.5 c.c.	B	Sick; recovered
1	11	1.0 c.c.	C	Sick; recovered
1	12	1.5 c.c.	C	Sick; recovered

The most toxic filtrate was obtained from lactose broth. It was then questioned whether the amount of sugar influenced the toxicity. To determine this point lactose broths of various strengths were made up, and the cultures grown and treated as already described.

Broth A contained .25 per cent lactose.
Broth B contained .5 per cent lactose.
Broth C contained 1 per cent lactose.

The guinea pigs used weighed from 200 to 225 grams.

TABLE X.

COMPARATIVE TOXICITY OF LACTOSE BROTHS.

DATE	G.P.	AMOUNT INOC., I.P.	BROTH	RESULT
Feb. 14	1	0.5 c.c.	A	Death in 5 hours
14	2	1.0 c.c.	A	Recovered
14	3	2.0 c.c.	A	Death in 48 hours
14	4	0.25 c.c.	B	Death in 7 hours
14	5	0.25 c.c.	B	Death in 12 hours
14	6	0.5 c.c.	B	Death in 3½ hours
14	7	1.0 c.c.	B	Death in 12 hours
14	8	2.0 c.c.	B	Recovered
14	9	0.5 c.c.	C	Recovered
14	10	1.0 c.c.	C	Recovered
14	11	2.0 c.c.	C	Recovered

Broth B, containing .5 per cent lactose, was most toxic, and this strength was used in the rest of the work with the filtrates.

The minimum lethal dose was determined, using varying quantities of the filtrate. One-tenth c.c. was found to be the smallest amount that would kill a 200-250 gm. guinea pig.

TABLE XI.

DETERMINATION OF THE MINIMUM LETHAL DOSE.

DATE	G.P.	WEIGHT IN GRAMS	INOC. I.P.	RESULT
Mar. 12	1	176	0.1 c.c.	Death in 20 hours
12	2	200	0.1 c.c.	Recovered
12	3	203	0.1 c.c.	Death in 9 hours
12	4	208	0.1 c.c.	Death in 3 hours
12	5	216	0.1 c.c.	Death in 4 hours
12	6	224	0.1 c.c.	Recovered
12	7	197	0.01 c.c.	No effects
12	8	180	0.01 c.c.	No effects
12	9	203	0.01 c.c.	No effects
12	10	220	0.01 c.c.	No effects
12	11	238	0.01 c.c.	No effects
12	12	248	0.01 c.c.	No effects

Peculiarities early noticed regarding the toxin were: (1) Small doses invariably were more fatal than large. (2) Death occurred in a few hours. Thus a guinea pig receiving a dose of 0.1 c.c. would die in from 3 to 24 hours, whereas one receiving 1.0 c.c. or more would, in many cases, recover. The M.L.D. of this toxin can not very well be compared to the M.L.D. of either diphtheria or tetanus toxin, since the lethal period is so short.

HEATED FILTRATES.

The toxicity of the filtrate demonstrated, it remained to determine whether the toxin was destroyed by heat,—in other words, whether the toxin was thermolabile or thermostabile. On March 21 a sterile filtrate was heated in a boiling water bath for ten minutes, cooled, and then injected into guinea pigs, intraperitoneally.

G.P. 1 Given 0.5 c.c. Death in 8 hours.
G.P. 2 Given 0.5 c.c. Death in 5 hours.
G.P. 3 Given 0.5 c.c. Death in 4 hours.
G.P. 4 Given 1.0 c.c. Death in 4 days.
G.P. 5 Given 1.0 c.c. Recovered.
G.P. 6 Given 1.0 c.c. Recovered.

The three pigs which had received 0.5 c.c. died within 8 hours, while only one out of three which had received 1 c.c. died, and that one in four days. Suspicion fell upon the use of the tricresol in the culture. Controls were, therefore, run using amounts even larger than in the cultures; but no ill-effects were ever observed. It was concluded that the toxicity was caused by the bacterial products, and was definitely proved by using filtrates to which tricresol had not been added.

On March 28 a sterile filtrate was divided into three parts: Part A was the normal, untreated filtrate; part B, the filtrate kept in a boiling water bath for 15 minutes; and part C, the filtrate kept in a boiling water bath for 60 minutes. When cooled to room temperature all three portions were injected, I.P., in varying amounts, into guinea pigs. The weights of the guinea pigs were, as in all this work, between 200 and 250 grams.

TABLE XII.

TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Death in 4½ hours
2	0.25 c.c.	A	Death in 7½ hours
3	0.5 c.c.	A	Death in 5½ hours
4	0.25 c.c.	B	Death in 4½ hours
5	0.5 c.c.	B	Death in 18 hours
6	1.0 c.c.	B	Death in 8½ hours
7	0.25 c.c.	C	Death in 30 hours
8	0.5 c.c.	C	Death in 18 hours
9	1.0 c.c.	C	Death in 4½ hours

TABLE XIII.

TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Recovered.
2	0.25 c.c.	A	Death in 5 hours
3	0.5 c.c.	A	Death in 5 hours
4	0.5 c.c.	B	Death in 18 hours
5	0.5 c.c.	B	Death in 5 hours
6	1.0 c.c.	B	Death in 4½ hours
7	0.5 c.c.	C	Death in 4 hours
8	0.5 c.c.	C	Death in 6 hours
9	1.0 c.c.	C	Death in 20 hours

As can be seen, the poison was not destroyed by heating, at boiling temperature, for one hour. This was corroborated by another series of tests, carried out as above, on April 10. The only change was made in the reaction of the media, where a −1 (1 per cent alkaline to phenolphthalein) was used instead of the neutral.

The postmortem findings were, as a rule, similar to those observed in the guinea pigs dying from the virulent culture. Marked inflammation of the intestines was characteristic in all animals dying from the filtrate, heated or unheated.

EFFECT OF FILTRATE UPON CATS AND RABBITS.

The sterile filtrate, when injected into cats, gave the same symptoms as the virulent culture, but was without fatal effects. The temperature increased as with the virulent culture.

February 15. Cat 7, weight 2250 gm., was given, I.P., 9 c.c. of a sterile filtrate from a .5 per cent lactose broth culture. Retching, vomiting, and purging took place within 45 minutes. The temperature readings were as follows:

Temp. at time of injection,	8.30	A.M.	37.0
	10.00	"	38.82
	12.00	"	39.64
	2.00	P.M.	39.82
	8.00	"	38.46

The cat recovered.

February 16. Cat 8, weight 2700 gm., was given, I.P., at 8.30 A.M., 9 c.c. of a 0.5 per cent lactose broth filtrate. Retching, vomiting, and purging occurred within 35 minutes.

Temp. at	8.30	A.M.	38.4	1.30	P.M.	40.42
	9.30	"	38.8	2.30	"	40.12
	10.30	"	39.0	3.30	"	40.28
	11.30	"	40.1	4.30	"	39.80
	12.00	"	40.1	5.30	"	39.3

The cat recovered.

A rise in temperature was the only effect produced in a rabbit injected with the filtrate.

February 15. Rabbit 180 was given, I.P., 5 c.c. of a 0.5 per cent lactose broth filtrate, at 8:30 A.M.

Temp. at	8.30 A.M.	38.5	11.45 A.M.	40.0
	9.00 "	40.02	1.45 P.M.	39.4
	9.45 "	39.70	8.00 "	38.08
	10.45 "	39.54		

The rabbit recovered.

FILTRATE FROM USCHINSKY'S SOLUTION.

It was considered that if the toxin had a definite chemical composition its study could be facilitated if it were formed in a synthetic medium. Uschinsky's medium was used. After inoculation with the organism and incubation for 7 days at 37° C., the solution was filtered through a Berkefeld candle and tested for sterility. Guinea pigs, injected with the filtrate, however, were not affected. This was not surprising in view of earlier work with cultures of the organism grown in this solution, when it was found that comparatively large doses were necessary to cause death in guinea pigs.

IDENTITY OF THE SOLUBLE POISON.

Attempts were made to isolate the soluble poison by the precipitation method, as with the toxins of diphtheria and tetanus; none, however, succeeded. The identity of the toxic agent remained, therefore, undetermined.

AGGLUTINATION.

The classification of the bacillus isloated from the poisonous cheese, from the study made thus far, would seem to place it in the B. coli group. The formation of a thermostabile poison, however, indicates that the organism is not of the true colon type. To determine, if possible, whether a more accurate classification could be made, agglutination tests were carried out. Rabbits were immunized to B. enteritidis, B. colon, B. paratyphoid A, B. paratyphoid B, and Bacillus 3B, and their sera used in the tests.

TABLE XIV.

AGGLUTINATION OF BACILLUS 3B BY IMMUNE COLON, ENTERITIDIS, PARATYPHOID A, AND PARATYPHOID B SERA.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
Immune colon serum									
B. colon	++	++	++	++	++	++	++	++	++
B. 3B	++	++	++	++	++	+	−	−	−
Immune enter. serum									
B. Enteritidis	++	++	++	++	++	++	++	++	+
B. 3B.	++	++	+	−	−	−			
Immune Para. A serum									
B. paratyphoid A	++	++	++	++	++	++	++	++	++
B. 3B	+	∓	−	−	−				
Immune para. B serum									
B. paratyphoid B	++	++	++	++	++	++	++	+	−
B. 3B	++	++	+	−	−	−			

TABLE XV.

AGGLUTINATION OF B. COLON, B. ENTERITIDIS, B. PARATYPHOID A, B. PARATYPHOID B., AND B. 3B, BY IMMUNE 3B SERUM.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
B. colon	++	++	++	+	–	–			
B. enteritidis	++	+	–	–					
B. paratyphoid A	+	–	–	–					
B. paratyphoid B	++	–	–	–					
B. 3B.	++	++	++	++	++	++	++	++	++

A study of Table XIV shows that the bacillus isolated from the cheese, designated as B.3B, was agglutinated by immune colon serum in a dilution of 1/300; by immune enteritidis serum, in a dilution of 1/50; by immune paratyphoid A serum, in a dilution of 1/10; and by immune paratyphoid B. serum, in a dilution of 1/50. The bacillus, therefore, belongs to the colon group. This is further corroborated by the data of Table XV. Of the four organisms (exclusive of B.3B) tested, B. colon was agglutinated in the highest dilution by the immune 3B serum. That the dilution did not exceed 1/100 was somewhat surprising in view of the fact that immune colon serum agglutinated the 3B bacillus in a 1/300 dilution. The relationship of the organism, as shown by its agglutination, to the enteritidis and partyphoid B. bacilli, may explain its formation of a soluble thermostabile poison.

It is to be regretted that no agglutination test could be carried out with the sera of the patients, none being available. Such tests have been carried out by several investigators. Hughes and Healey,[26] in their report of the Aldershot epidemic, stated that a colon bacillus isolated from the liver and kidney from a fatal case gave no reaction with the blood sera from other cases. No reaction was also given by the blood sera from several of the cases with Gärtner's bacillus. Berg[28] isolated the B. Paratyphoid B from the intestines and organs from a fatal case of cheese poisoning. This bacillus was agglutinated by the blood serum of the wife of the dead man, herself a victim of cheese poisoning. Fonteyne[18] found that samples of blood taken ten days after the accident did not agglutinate the bacillus he had isolated from the cheese. This bacillus was not agglutinated by sera immune to B. Aertrycke, B. de Bruges, and B. Typhoid. Serum immunized to the cheese bacillus agglutinated B. Aertrycke in 1/100 dilution; B. de Bruges, in 1/10 dilution; and B. Typhoid, in 1/250 dilution.

IMMUNITY.

In the immunization experiments, guinea pigs, on account of their susceptibility and adaptability to this kind of work, were used in preference to other laboratory animals. Immunization was obtained most satisfactorily by vaccine treatment. Agar slant of the organism, of 24 hours' growth, were washed with physiological salt solution, filtered through sterile cotton, heated at 58° C. for 1 hour, tested for sterility, and then standardized. Such a vaccine standardized on December 12 was found to contain 400,000,000 organisms per cubic centimeter. On December 13, six guinea pigs weighing from 200 to 220 grams each, were given, I.P., 5 c.c. of this vaccine. On December 17 a second dose of 1 c.c. was given; while the third dose, 1 c.c., was given on December 21. On December 26 all six pigs were given 1 c.c. of a 24 hour culture of Bacillus 3B. None

of the pigs died. A control guinea pig, injected with the same amount of the organism, died within 5 hours.

On February 4, 8 guinea pigs, weighing from 200 to 225 gm., were injected with .5 c.c. each of vaccine, made as above, and standardized so that 1 c.c. = 250,000,000. A second injection of the vaccine, 1 c.c. was given on February 8. One of the pigs died on the following day. The third and final injection was given, in 1.5 c.c. amounts, on February 12. On February 19 all were given 1 c.c. of a 24 hour culture of Bacillus 3B. One guinea pig died on the 20th; the rest were unaffected. A control guinea pig, injected with .5 c.c. of the virulent culture, died in 16 hours. These tests show that a high degree of immunity is obtainable by vaccine treatment.

SERUM IMMUNITY.

The serum of guinea pigs immunized by several injections of vaccine, when injected into normal guinea pigs, immunized them to fatal doses of the virulent culture.

March 3. Two immune pigs were bled and their sera obtained aseptically. Four guinea pigs (210-225 gm.) were injected, I.P., with 0.1 c.c. of the immune serum. On March 8 one of the pigs was injected with 1 c.c. of a 24 hour lactose broth culture of B.3B. Death occurred 3 days later. A control pig died in 12 hours. On March 12, the second and third pigs were injected with 1 c.c. and 1.5 c.c., respectively, of a 24 hour lactose broth culture of B.3B. Guinea pig 3 died on the sixth day, while guinea pig 2 was unaffected. A control died in 12 hours.

Two guinea pigs injected, March 3, with .25 c.c. of the immune serum, were injected, on March 12, with 1 c.c. of a virulent culture of Bacillus 3B. Both were unaffected. A control died in 9 hours.

IMMUNITY TO SOLUBLE POISON.

While it was comparatively easy to obtain immunity to the virulent organism, it was very difficult to obtain immunity to the soluble poison it elaborated. Invariably the guinea pigs would die after the second or third inoculation, regardless of whether these doses were increased or decreased.

Thus, for example:

On March 12, three guinea pigs (200 gm.) were inoculated with .01 c.c. of a sterile lactose broth filtrate. On March 16 each received a dose of .1 c.c. of the filtrate. One died on the second, one on the third, and one on the fourth day after the inoculatton. Again, on March 12, three guinea pigs were inoculated with .001 c.c. of the filtrate. On March 16 a second injection of .01 c.c. was given. One pig died on the 20th. The two remaining pigs were inoculated on the 24th, with .5 c.c. of the filtrate. These, as well as a control, died in 4 hours.

On March 21, six guinea pigs (200-250 gm.) were injected with .01 c.c. of the filtrate. On March 26, a second injection, .005 c.c. was given. One pig died on the following day, and one on the 29th. A third injection of .02 c.c. was given on April 7. One pig died on April 8. On April 14 the three remaining pigs and a control were injected with .25 c.c. of a freshly prepared filtrate. One of the pigs and the control died, the former in two days and the latter in seven hours. The other two guinea pigs were unaffected.

Guinea pigs immunized to fatal doses of the poison are immune to fatal doses of the virulent organism. Injections of heated sterile filtrate will immunize guinea pigs to fatal doses of the organism.

Two guinea pigs were injected, I.P., on March 3 with .5 c.c. of a sterile .25 per cent lactose broth filtrate. On March 12 they were given 2 c.c. each of .5 per cent lactose broth filtrate. The third dose, .2 c.c. of a .5 per cent filtrate, they were given on the 16th. On March 24 they were both injected with a freshly prepared .5 per cent filtrate, each receiving

.5 c.c. They were unaffected. A control died in 4 hours. On March 30 both were given 1 c.c. of a 24 hour culture of Bacillus 3B. They were unaffected, while a control, given the same dose, died in 15 hours.

On March 28 a .5 per cent lactose broth filtrate was heated, for one hour, in a boiling water bath. Varying quantities were injected into guinea pigs.

G.P. 1, weight 300 gm., received .25 c.c. No effects.
G.P. 2, weight 313 gm., received .50 c.c. No effects.
G.P. 3, weight 330 gm., received 1.0 c.c. Sick; recovered.
G.P. 4, weight 313 gm., received 1.5 c.c. Sick; recovered.

On April 8 all four received .5 c.c. of the heated filtrate. No effects were observed in any of the animals. On April 17, they were injected, in varying doses, with a 24 hour lactose culture of Bacillus 3B.

G.P. 1 received 2 c.c. of the culture.
G.P. 2 received 1.25 c.c. of the culture.
G.P. 3 received 1.0 c.c. of the culture.
G.P. 4 received 1.0 c.c. of the culture.

All were sick, but recovered on the following day. A 350 gm. control pig, given 1 c.c. of the culture, died in 10 hours.

From the data given it is evident that immunity can be obtained by injections of the sterile filtrate not only to the soluble poison itself, but also to the virulent culture of the organism. The soluble poison is undoubtedly not a true toxin, but evidence has been given that it does form immune antibodies.

CONCLUSIONS.

The data and results obtained in this work warrant the following conclusions.

1. Six cases of cheese poisoning were caused by a toxicogenic bacillus.
2. Morphological and biochemical reactions, and agglutination tests prove that the bacillus belongs to the colon group.
3. That it is not a true colon bacillus is shown by its formation of a soluble thermostabile poison.
4. Poisoning can not be induced in animals either by feeding the poisonous cheese or by feeding with cultures of the isolated organism.
5. Mice, guinea pigs, rabbits, cats, rats, and dogs are susceptible, in the order named, to the bacillus.
6. The bacillus does not form tyrotoxicon.
7. Five-tenths per cent lactose broth, neutral to phenolphthalein, is the medium most suitable for the production of the poison.
8. The soluble thermostabile poison produced by the bacillus is a toxin-like body, of unknown chemical constitution.
9. Immunity to the organism can be acquired by vaccine and immune serum injections. It can also be obtained by injections of the sterile, cell-free filtrate from lactose broth cultures.
10. A lesser but yet marked protection can be acquired by injections of alkaline ether extracts of whey from milk cultures.

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FURTHER EXPERIENCE IN THE TREATMENT OF TYPHOID FEVER BY THE INTRAVENOUS INJECTION OF SENSITIZED TYPHOID VACCINE SEDIMENT*

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A LITTLE over a year ago Chickering and I[1] reported on the treatment of over fifty cases of typhoid fever by the intravenous injection of typhoid vaccine, which results, with some additional cases, were presented before this Association a year ago. Since that time opportunities have been available for the study of two additional groups of cases: the first at the Presbyterian Hospital in New York City, made possible through the great courtesy of Warfield T. Longcope; and the second a group of twenty-eight cases occurring in the Providence Hospital, Oakland, California, and rendered available for this study by the kindness of Edward von Adelung. The latter group of cases is of particular interest not only as being considerable in number, but as giving us our first opportunity of dealing with epidemic typhoid. These twenty-eight cases occurred among a group of workmen employed in constructing the San Pablo dam in Contra Costa county, and were due to a well traced contamination of drinking water which led to the occurrence of some fifty-two cases of typhoid fever, that have been reported on from the epidemiological standpoint by Geiger, Macmillan, and Gillespie.[2]

The method we have employed in treating typhoid has already been detailed, but may be repeated in essentials here. Treatment has consisted in the intravenous injection of the Gay-Claypole sensitized vaccine sediment, a preparation obtained from multiple strains of the typhoid bacillus of local and general origin, so chosen as to represent the characteristic antigenic groups of the typhoid bacilli that have been tentatively separated by Hooker, whose work has since been confirmed by Weiss. The vaccine is further prepared by treating these strains of typhoid bacilli with a rabbit immune serum of high agglutinating titer, subsequently washing them, and then killing and precipitating them by means of absolute alcohol. This killed vaccine is then dried to constant weight and ground in a mechanical mortar until the bodies of the bacteria are thoroughly disintegrated. The endotoxic substances are extracted by means of carbolated saline and the supernatant fluid rejected, the sediment of ground bacterial bodies being alone employed for injection. The use of this sensitized vaccine sediment has been reported on elsewhere in reference to its use in prophylactic immunization.[3] It has been found that this vaccine may be injected intravenously in adults in doses beginning with 0.02 of a milligram and gradually increasing in some instances to 0.06 of a milligram, without any harmful effects, although such injections give rise to a characteristic train of symptoms which seem intimately connected with the favorable results obtained. Our experiments herewith reported represent 1200 injections, and it may confidently be stated that no harmful results in the cases of

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typhoid fever involved, either immediate or delayed, are referable to the vaccine.

A synopsis of the cases of which this is a report is given in Table I, which we may proceed to explain in some detail.

TABLE I.

Total cases examined	194
Nontyphoid cases	62
Cases of typhoid on clinical basis alone	8
Cases of proved typhoid	124
Cases of proved typhoid not treated	26
Cases treated as suggested	98

One hundred ninety-four cases of suspected or possible typhoid were examined by full laboratory methods, and of these cases 62, by agglutination tests, blood cultures, and, in some instances, stool and urine examinations, could not be identified as typhoid fever. All of these sixty-two cases could eventually be likewise dismissed on clinical evidence as well, as certainly not typhoid. There remain, however, eight cases which may have been typhoid fever on the basis of the clinical examination, which, however, it was impossible to confirm by laboratory tests; these cases are not included in our series, although two of them were treated in the manner described with beneficial results. There remain, then, 124 cases of typhoid fever that may be accepted as certain on the basis of both clinical and thorough laboratory examination. Every case of proved typhoid fever, with the twenty-six exceptions to be mentioned presently, was treated in accordance with the method to be fully outlined at a later point.

We should here perhaps explain and possibly defend our decision to treat every available proved case. It has been a frequent custom among clinicians who wish to investigate any new method of therapy to treat a certain number of cases in a given series by the method in question, leaving another group of cases of variable size under the routine previously adopted for the object of furnishing what have been regarded as controls. This procedure has been patterned after the methods ordinarily in vogue in animal experimentation, where truly controlled experiments are essential and convincing. It should be pointed out, we believe, that no group of human cases of typhoid fever or of any other disease can so be utilized except on the basis of a relatively large number of cases, where purely statistical methods of computation may be employed. Chance infection in typhoid fever in no way represents the methods of experimental precision which are possible in animal experimentation, and no two cases of typhoid fever exactly resemble one another. In the first place, the infecting dose when derived from various sources of contagion must be extremely variable, and even in the case of an epidemic, as in the San Pablo epidemic, which forms a part of our series and where the contaminating dose may be assumed to be somewhat similar in all the cases, the widest variation is found in the individual reaction to the infection, a variation based on the individual resistance natural or acquired. We regard, then, the use of alleged control cases in a study of this sort as fallacious, inasmuch as they do not really control at all, and should prefer to judge our results, first, on the basis of what actually occurs in each and every case following the treatment employed, and, secondly, by comparison of this group of cases, although limited, with other recognized standards in respect to mortality incidence, dura-

tion of the disease, and the percentage of occurrence of complications. We believe that judgment arrived at on this basis requires more convincing evidence, which we believe is fortunately present in this particular case.

Twenty-six of our cases of proved typhoid were not treated, either because it was impossible to treat them, or because we believed that any results obtained in them might lead to an incorrectly favorable interpretation. Thus, in nine of our cases the temperature had already begun to fall, or the patient was indeed already afebrile. Three of them really represented a post-typhoidal condition, osteomyelitis, for example. Five refused treatment. In three, intravenous injections could not be given. Concerning three, no record is obtainable, and in three more death occurred before the laboratory diagnosis had been arrived at.

It should be emphasized that in no case was treatment withheld on account of the seriousness of the patient's condition, a fact of significance in consideration of the low mortality obtained. In two at least of our six fatal cases the prognosis seemed inevitably fatal when treatment was begun.

We may consider for a moment the results of laboratory diagnosis in the ninety-eight cases that were actually treated. The diagnosis was based first on the agglutination test (Widal) in all instances, on blood cultures in ninety-four of the ninety-eight cases, and in many instances on detection of the typhoid bacillus in the stool and urine. The agglutination tests were made by the macroscopic method with the employment of formalin-killed cultures of the typhoid bacillus. We have stated in our previous communication that complete sedimentation of the added bacteria overnight, even in dilutions as low as one to ten, are, if not absolutely diagnostic of typhoid fever (or typhoid vaccination), at least strongly presumptive evidence of it. We may mention here that of sixty-seven control, nontyphoidal cases tested in connection with this series, the Widal was negative by this method in a dilution of one to ten in sixty-four instances (95.5 per cent). The three exceptions were liver abscess, bronchitis, and trichiniasis, in the first and last of which previous typhoid could by no means be ruled out. On the basis of the lower dilution 94.8 per cent of our cases gave a positive Widal at some time during the disease, or if a higher and usual standard of dilution of one to forty alone is acceptable, 91.8 per cent of our cases were positive. Blood cultures (made by adding 10 c.c. of blood to 200 c.c. of 10 per cent bile broth) were positive in our series in 71 per cent, and in 13 per cent only were blood cultures negative when more than one examination had been made. In 68 per cent of the cases both Widal and blood cultures were positive. In two cases alone of the ninety-eight were both Widal and blood cultures negative, in which instances diagnosis was based on the isolation of the typhoid bacillus from the stools or urine.

The intravenous injection of our vaccine produces a series of distinct symptoms which vary markedly in intensity with individuals and with the dose employed. The usual amount on initial injection has been 1/50 of a milligram (corresponding to 150 million bacteria), and a corresponding dose in children, who, as in prophylactic immunization against typhoid, react less markedly than adults to corresponding amounts. I shall later refer to the findings of other investigators who have used other vaccines intravenously and who have described

alarming or even dangerous symptoms following their employment. It should be noted, however, that our vaccine, owing to its sensitization and the removal of endotoxins, certainly gives less perturbing, and, so far as we have observed, no really untoward effect. It seems necessary to produce a moderate reaction in order to effect the desired result, and the dosage in successive inoculations has been increased slightly in order to produce a similar train of symptoms on each inoculation, which a continuance of the same dose usually fails to do. The injection of the vaccine intravenously is followed in from fifteen minutes to an hour by a chill, which lasts from a few minutes to ten or fifteen minutes. This chill, or shaking, is not accompanied by a feeling of coldness, but rather by a sense of involuntary, spasmodic, muscular contraction. The chill is accompanied by a rise in temperature of one to three degrees, which reaches its height within three hours after injection and then falls. There may be a rise of temperature without a chill or the reverse. The rise in temperature is accompanied by a leucopenia, which may fall as low as 2000 to 3000 to the cubic centimeter. The chill is accompanied by an increase in the pulse rate and may be accompanied by slight cyanosis, slight respiratory distress, and frequently a sense of discomfort.

The temperature reaches normal or subnormal in about twelve hours. This fall in temperature is accompanied by sweating, which may be profuse and last for several hours, relaxation, and usually general amelioration of such symptoms as headache, delirium, and the like. The patient often feels perfectly well, and this condition, even when transitory, seems beneficial. Coincident with the fall in temperature there occurs a rise in the leucocytes, which may reach as high as 40,000 and which are characterized by a relative polymorphonuclear increase. I shall later refer, in reviewing the work of others with similar methods, to the dangers that have appeared with slightly different vaccine preparations, and to the contraindications to such injections that undoubtedly exist. It may be repeated, however, that in our experience of 1200 injections reactions of this sort have never appeared harmful, either immediately or ultimately, and contraindications seem, in the light of increasing experience, to grow progressively fewer.

No detailed method of procedure can be prescribed for treating any given case of typhoid fever by this method. The best results seem to be obtained by provoking a distinct, but not too severe, reaction of the type outlined. The dose necessary to produce such a result varies markedly with the individual and the particular balance already established between the typhoid bacillus and the reaction antibodies in the host. The temporary drop of temperature to normal may become permanent and remain there, in which case no further injections are required, except for the prevention of relapse. If the temperature again rises over a period of two or three days, the injection should be repeated in slightly increased amount, and so on until the desired result is produced or further injections are judged futile. A considerable number of injections may be given with perfect safety. As many as fifteen or sixteen have been given in certain instances, but if no striking result is obtained following three or four injections at two or three day intervals, very little effect from the treatment may be expected.

It has seemed wise to us previously to separate our cases into three rather definite groups in respect to the results produced by the vaccine injections, and we see no reason to change this grouping in the light of further experience. We believe that this grouping, based on results produced, is a conservative one and by no means represents all the benefit that attends this method of treating typhoid fever. We have briefly referred to the fact that the temperature

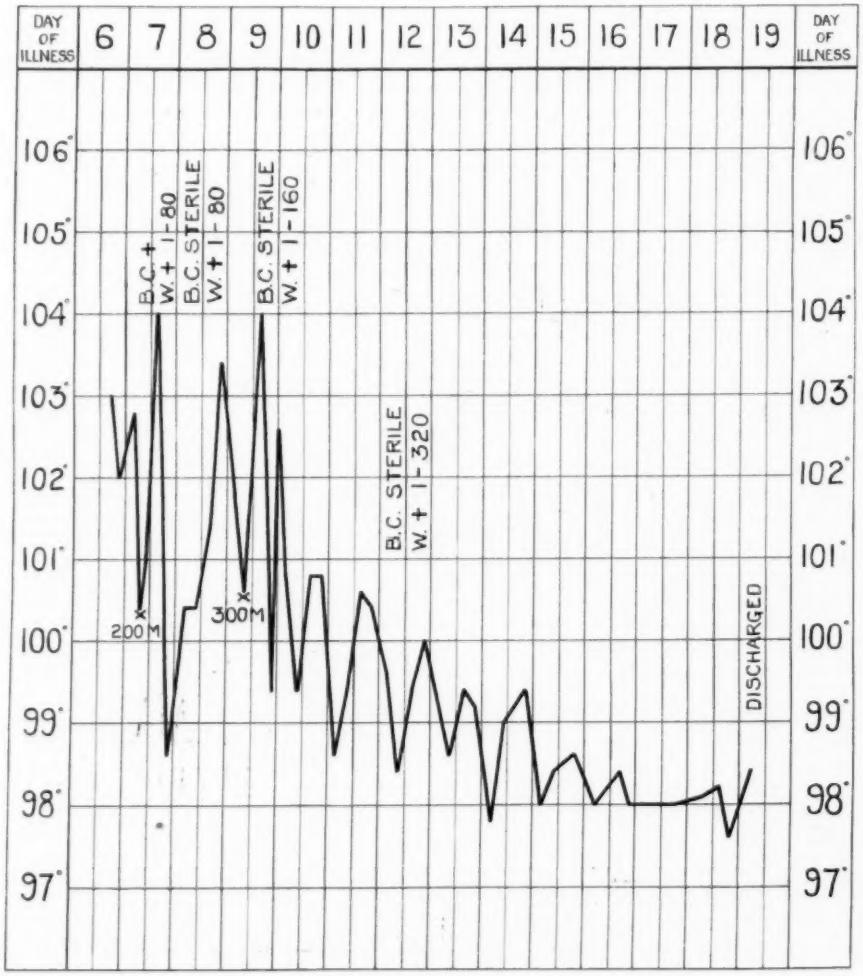

Fig. 1.—Case No. 35. Illustrating an abortive cure.*

excursion with return to normal is accompanied by subjective feelings of well being, although at times of slight weakness, and would seem to be both immediately and ultimately of benefit to the patient. Such subjective amelioration is, of course, open to error in interpretation, but would seem, not only to us but

*The data in these charts (Figs. 1, 2, 3) are abbreviated as follows: B. C., Blood Culture; W. Widal; A. T., Antityphoid serum; 75 M, Sensitized vaccine corresponding to 75 million typhoid bacilli. The temperatures indicated are the highest maxima forenoon and afternoon on each day by rectum.

to others who have watched our cases, to be frequent and of a convincing nature. The nurses, for example, in charge of the cases not only volunteer remarks as to the benefit produced by the vaccine injections, but emphasize the lessened care which patients treated in this manner entail. We prefer, however, to base our judgment as to the good results of the method rather on the more objective results that have been produced, the shortening of the duration of the fever, its frequent abrupt termination in an abortive form, the lessening of mortality and of complications in particular. We have classified the three groups of results obtained as abortively recovered, as benefited, or as unaffected,

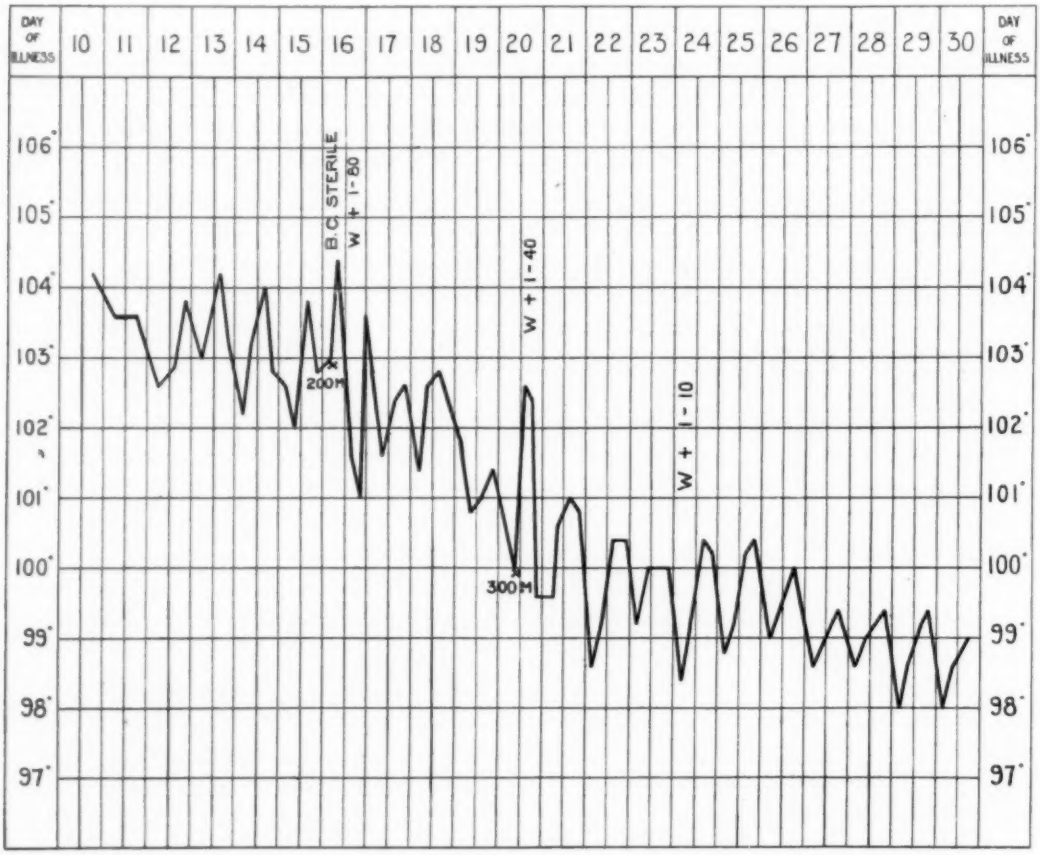

Fig. 2.—Case No. 43. Illustrating a benefited case.

and herewith present again charts from our previous article illustrating types of recovery of these three kinds. (Figs. 1, 2, and 3.)

A summary of our ninety-eight cases classified under these three headings proves of interest not only in respect to result achieved, but as bearing on the mechanism by which it is effected. In Table II are expressed certain characteristic findings in each of these groups of cases under consideration, which we may briefly refer to in drawing certain conclusions as to their significance.

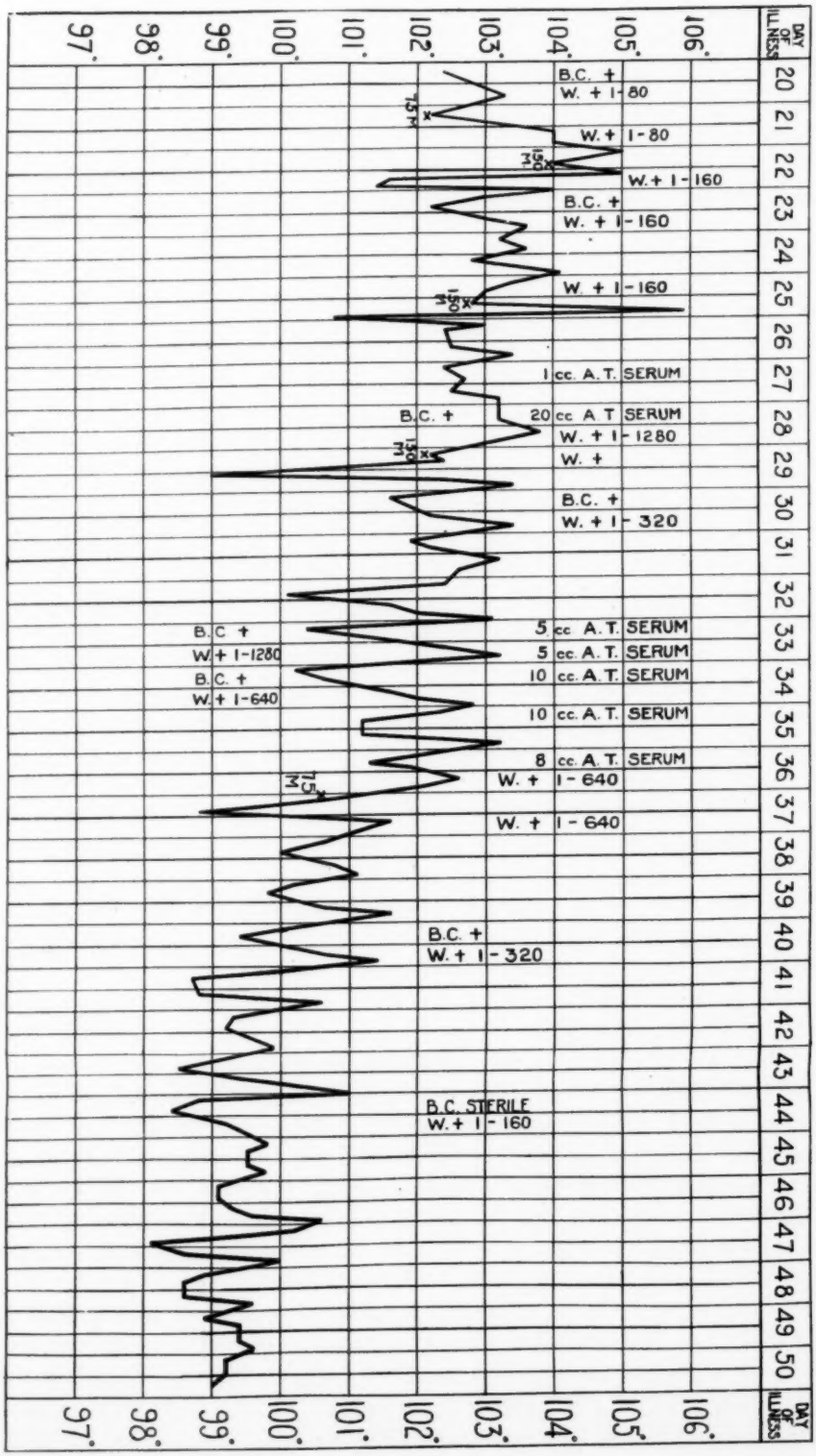

Fig. 3.—Case No. 47. Illustrating an unaffected case.

TABLE II.

SUMMARY OF RESULTS IN 98 CASES OF TYPHOID TREATED BY INTRAVENOUS INJECTION OF SENSITIZED VACCINE SEDIMENT.

	No.	Age	Widal Titer	B.C.	Treatment begun Day	No. of Treatments	Permanent Normal Temperature	Duration of Treatment
Aborted	33	26.2	296.0	36.6%	13.4	1.88	20.4	7.0
Benefited	32	24.2	156.5	70.9%	14.8	3.20	30.6	15.8
Unaffected	33	28.8	114.8	84.8%	13.7	4.85	46.8	33.1

In the first place, it will be seen that our cases may be divided into three almost exactly equal groups in respect to results produced. These groups, moreover, are of almost identical age, and it is also found that treatment was begun on the average at practically the same time in the three groups. They differ in practically all other respects. We find in the first place that antibody formation, as evidenced by the Widal or agglutinin titer in the cases before injections were begun, varies markedly in accordance with the eventual outcome of the case in question. In the abortively cured cases the Widal is found to be on the average over twice as high as in the unaffected cases, whereas the benefited cases lie intermediate between the two groups. A strong Widal is evidence, then, of a good prognosis, not only in respect to these treated cases, but probably also in untreated cases, to judge from the experience of others. Inversely correlative with the strength of the Widal is the occurrence of the percentage of positive blood cultures in the three categories under discussion; whereas the cases that respond less well to treatment are found to give positive blood cultures in nearly 85 per cent of cases, those that recover abruptly on treatment had positive blood cultures in only 36.6 per cent. The benefited cases again lie intermediate between the two. These relations between blood cultures and antibody formation we have already referred to as representing a balance established in the patient before intervention between the infecting agent and the resisting power of the host. When the resisting power is strongly developed, a favorable outcome may be more confidently expected.

This group of cases, which have been classified as abortively cured or abortively recovered, suggest in some respects the mild or abortive typhoid cases which are discussed in most extensive monographs on this disease. The typhus levissimus of Griesinger, the typhoidette of Brouardel, or abortive typhoid, forms a well recognized group or groups that must occur in every considerable collection of cases of typhoid fever. Estimates vary as to the exact proportion of such cases, as may normally be expected. It is probable, as Curschmann[4] suggests, that at least two groups should be formed, the mild typhoid fevers and the abortive typhoid fevers, the latter more frequently, though by no means invariably, characterized by a somewhat abrupt rise and fall of temperature and an abbreviated fastigium. McCrae in his 1500 cases found three per cent of mild forms of the disease, and, in addition, 0.1 of one per cent of his cases recovered by crisis. Letulle[5] found that these two types of the disease occurred particularly in certain epidemics and in children. He finds something over 17 per cent in the cases of the disease which he collected.

This figure may certainly be regarded as an extreme maximum of occurrence. According to Curschmann, the abortive and mild forms frequently have only three to six days of fever and almost never over ten or twelve days, with which statement Hare and Beardsley[6] would agree. At all events, the cases of mild and abortive typhoid together must constitute a relatively small percentage of all, in spite of the figures of Letulle. Hare and Beardsley note that Coleman found only twenty-four such cases in five years' experience at the Bellevue Hospital in New York. At all events, no such number as one-third of all the cases, as in our series, could be found in this way without some type of intervention. It may further be noted that our cases almost invariably have occurred beyond the limit of ten to twelve days set by Curschmann, and have practically invariably been characterized by a critical fall of temperature, and what is more important still, show a direct relation between this critical fall and the injection of vaccine. Our cases do not represent the mild epidemics of Letulle, nor has any considerable number of them been furnished by children. They have been gathered over three seasons and in widely different localities. As may be anticipated, the abortively recovered cases represent, by and large, the milder cases of the disease in this series as estimated before treatment was begun. Our characterization of a preponderance of these abortively cured cases as milder has no relation, however, to the small group of mild and abortive forms that have just been discussed. We have endeavored to register our impression as to the severity of each case before treatment was begun, and the results are expressed in Table III, where it is found that many more severe cases occur among those that remain unaffected by treatment, and many more mild cases occur among those that are aborted, whereas the benefited cases would seem to lie intermediate between the two.

TABLE III.

GENERAL CONDITION OF CASES BEFORE TREATMENT.

	SEVERE	MODERATE	MILD
Unaffected	26	6	1
Benefited	10	17	5
Aborted	4	13	16

It is evident, then, that the milder cases react better to treatment than the more severe, but even the most severe ones will in some instances at least be benefited and even aborted. We believe the effects actually produced are best represented by the figures given under "Permanent normal temperature" and "Duration of Treatment" in Table II. It is found that a permanent normal temperature was obtained in the third of the cases that are called aborted on an average at about the twentieth day. When one considers that the treatment of these cases was not begun until the thirteenth day, the really significant figure of seven days as the average duration of treatment required to restore the patient to an essentially normal condition, so far as the temperature is concerned, is obtained. We find, moreover, that it took on the average a little under two injections to produce this result. The fall of temperature by crisis, which is exemplified in the Fig. 1 used to illustrate the abortive cure, is characteristic of

this category, although the abrupt fall may not occur in every instance on the first injection.

The benefited cases, again, are well typified in Fig. 2; each successive injection of vaccine produces a permanent fall in temperature on the average of one degree, and it may require several, on the average a little over three injections, to reduce the temperature to a permanent normal. The average duration, then, of these cases is found to be 30.6 days, and the duration of treatment the more significant figure, 15.8 days.

The cases that have been listed as unaffected, although showing the ordinary temperature excursions following each injection, are characterized by a prompt return of the temperature to its original or nearly its original height. The impression has remained in the minds of many who have seen these cases that they are really benefited, but since we have taken the reduction of temperature as our criterion of results, we prefer not to emphasize any good effect that may have occurred in these cases, beyond the diminution in complications and mortality, to which we shall presently refer. Even in this group of cases the average duration is by no means extraordinary, 46.8 days, although it does exceed somewhat, perhaps, a normal average duration of cases of typhoid fever. It is extremely difficult to estimate what such an average duration usually is. Curschmann regards the mild cases as lasting on an average of twenty-one to thirty-three days. In McCrae's[7] figures the average febrile period was thirty-one days. It should be noted that our figures refer to the total duration of the disease from its initial symptoms, so far as they can be estimated from the patient's history, and not from the known duration of the fever itself. The group of cases, then, that was best affected may reasonably be regarded as having run a distinctly short course, and the average of all cases, which is about twenty-nine days, is not markedly different from McCrae's thirty-one days.

The mortality in this series of ninety-eight treated cases is 6.6 per cent, which, when one considers that the cases were treated over a period of three seasons under varying conditions of care in private houses, as well as hospitals, and, moreover, treated in different parts of the country, is certainly a very satisfactory figure. The average mortality is usually figured at 10 per cent. The cases of death were predominantly due to the accidents of typhoid, three to hemorrhage, complicated with perforation in two instances, two to a combination of laryngitis and bronchopneumonia, and one only to typhoid toxemia.

The complications, again, were few in number, and only thirteen in all in the entire ninety-eight cases (13.2 per cent). This means, not that thirteen cases showed complications, for there were indeed only eight cases which showed complications, but the total number of complications was thirteen. These complications were as follows: pyelitis one case, lobar pneumonia one case, laryngitis two cases, bronchopneumonia two cases, toxemia one case, hemorrhage four cases, perforation two cases. The percentage of hemorrhage (4%+) and of perforation (2%) is certainly below what may be expected as the usual averages for these complications; 5 to 10 per cent for hemorrhage and 3 to 4 per cent for perforation. Although the earlier study of vaccine therapy in typhoid certainly led to the im-

pression, particularly when the vaccine was administered subcutaneously, that complications were diminished (Krumbhaar and Richardson,[8] Callison[9]), more recent work would seem to show that no distinct effect on the complications can be expected by the more recent intravenous type of treatment (Szecsy,[10] Wiltshire,[11] Reiter,[12] and Guinon and Malarte[13]).

The relapses are of particular interest in this series of cases because they show what we believe to be the effect of a method of treatment which we suggested in our previous article, and which has since been emphasized by others. It seemed from our previous work that relapses would be less likely to occur if the intravenous injections were followed, when the temperature had returned to normal, by a set of three subcutaneous injections on alternate days of the regular prophylactic dose (800 million bacteria or 0.1 milligram of our sensitized vaccine.)* This method of treatment has likewise been recommended by Meyer,[14] Meyer and Altstaedt,[15] and Wiltshire.[11] There were ten relapses (10.2 per cent) in our series which may be compared with the usual average of 11.4 per cent given by McCrae. Eight of our relapses, however, occurred in cases in which the intravenous injections were not followed up by the subcutaneous treatment, so that when we come to divide the cases in relation to this treatment into two categories, we find that relapses occurred in only 5.39 per cent of the subcutaneously treated cases (37) and in 11.59 per cent of the cases in which intravenous injection alone was given (69). We believe, then, that this method should be followed in every case.

SUMMARY OF RECENT LITERATURE ON VACCINE THERAPY OF TYPHOID.

We may next consider the very considerable number of articles which have dealt with different methods of vaccine treatment of typhoid fever in the past few years. We do not intend at this point to repeat a historical account of the development of vaccine therapy in this disease, but wish here to give a summary of the results in very recent years, roughly from 1913 to 1917, both as indicating the extent to which these investigations have been pursued, but more particularly as indicating which of these methods is best. We have summarized in Table IV the results obtained by a considerable series of observers who have used different, or in some cases several methods of vaccine treatment.

TABLE IV.†

SUMMARY OF RESULTS OBTAINED BY RECENT OBSERVERS (1913-17) IN THE TREATMENT OF TYPHOID FEVER BY VACCINES ADMINISTERED IN VARIOUS WAYS.

	OBSERVERS	TOTAL CASES	ESTIMATES BASED ON	BENEFITED	MORTALITY
Untreated vaccine subcutaneously	30	1001	512	46%	14.5%
Sensitized vaccine subcutaneously	14	593	239	69%	8.0%
Untreated vaccine intravenously	22	501	233	62%	13.0%
Sensitized vaccine intravenously	12	487	316	85%	11.0%

*These vaccine injections in the convalescent typhoid will in a few instances cause a rise in temperature of a degree on the same or the following day.

†The authors referred to in this summary are herewith given, a division being made in respect to the type of vaccine employed and its method of administration, as in the table. (1) Recent authors reporting

It will be noted, first, that the most frequent method of vaccine treatment in typhoid has been the subcutaneous injection of ordinary and usually heat-killed, untreated typhoid vaccines. The thirty authors on whose work we base our estimates, it should be repeated, are only very recent writers on the subject and by no means indicate the extent to which this method has been employed in previous years, a summary of which may be found in some of the articles quoted. This method of treatment, however, still remains somewhat the predominating one. Later modifications that have been introduced are, first ,the use of sensitized vaccine subcutaneously, and then the injection intravenously either of plain vaccine or of sensitized vaccine. We have endeavored to estimate the results obtained by each of these methods of treatment. It is obvious that any such estimate is arrived at with difficulty. In the first place, many authors give wholly indefinite accounts of even the number of cases that they have treated, and even when the number is mentioned, the results obtained are frequently referred to as good, bad, or indifferent. The figures in the third column, on which the estimates of the percentage of benefit derived from each method of treatment is based, represent all the cases in the total number considered, in which a definite statement of result by the author was made.

One thing stands out preeminently in a consideration of this total recent literature, and that is that the great majority of the authors believe the vaccine treatment, irrespective of the method pursued, to be of benefit. The very few individuals who remain unconvinced of the value of the treatment or mention dangers in its employment have based their opinion on very few cases and a corresponding inexperience in the use of vaccine (Zupnik,[16] Peutz,[17] Sladek and Kotlowski,[18] Deutsch[19]). It seems further distinctly indicated from this table that the degree of benefit, and, to some extent, the reduction of mortality, would seem to increase with each successive innovation that has been suggested in recent years. The best results unquestionably seem to have been obtained by the intravenous injection of sensitized typhoid vaccine, the sensitization having been either by the use of convalescent serum, as advocated by Ichikawa, or by the use of a vaccine similar to the Besredka living typhoid vaccine. The superior results of sensitized vaccines and of intravenous injection over other methods is further evidenced when we consider those authors who have used more than one of the methods outlined. Thus, we find that Löwy, Lucksch and Wilhelm[20] obtained better results in their cases by giving plain vaccine intravenously than when it was given subcutaneously. Similar conclusions were drawn by Ortiz, Acuna and Belloc.[21] Paltauf,[22] Holler,[23] and Feistmantel[24] all conclude that sensitized vaccines produce better results than unsensitized vaccines.

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on the use of untreated vaccine subcutaneously: 1911, Fletcher, 1912, Sadler, Callison; 1913, Fornet; 1914, Josne and Belloir, Weil, Sacquépée and Chevrel, Guinon and Malarte, Pensuti, Daretti; 1915, Goldscheider and Aust, Bourke, Evans and Rowland, Feistmantel, Mertz, Peiper, Krumbhaar and Richardson, Gröer, Wiltshire and MacGillycuddy, Löwy, Lucksch and Wilhelm, Kahn, Reiter, Meyer, F., Brach and Fröhlich, Boselli; 1916, Lemanske, Peutz, Whittington, Zupnik, Müller and Leiner, Waitzfelder. (2) Authors who have employed sensitized vaccine subcutaneously: 1912, Ardin-Delteil, Negre and Raynaud; 1913, Ardin-Delteil, Negre and Raynaud, Roques, Boinet; 1915, Petrovitch, Ardin-Delteil, Negre and Raynaud, Garbat, Feistmantel, Szecsy, Liebermann, Fellner, Löwy, Lucksch and Wilhelm, Boral, Deutsch; 1916, Galambos, Mayer. (3) Authors who have injected untreated vaccine intravenously: 1913, Thiroloix and Bardon; 1914, Kraus and Mazza, Kraus; 1915, Biedl, Ditthorn and Schultz, Csernel and Marton, Reibmayr, H., Mazza, McWilliams, H. I., Holler, Paulicek, Meyer, Rhein, Leutz, Ortiz, Acuna and Belloc; 1916, Faginoli, A., Petzetakis, Zupnik, Müller and Leiner, Löwy, Lucksch and Wilhelm. (4) Authors who have injected sensitized vaccine intravenously: 1912, Ichikawa; 1914, Meyer and Altstaedt; 1915, Biedl, Eggerth, Holler, Sladek and Kotlowski, Koramyi, Landsberger, Löwy, Lucksch and Wilhelm; 1916, Rohoni, Galambos; 1917, Caronia.

It remains now to offer what explanation we may as to the mechanism by which the unquestionably beneficial effects of vaccine therapy in typhoid are brought about, and, particularly, why the intravenous treatment is better and the sensitized vaccine superior to the unsensitized. These relative differences seem to emerge inevitably from the attentive consideration of the literature that we have given, and have, at least in part, been preceded or followed by theoretical explanations of heuristic value.

SPECIFICITY OF VACCINE THERAPY.

We were careful to state in our previous article that the method of therapy which we were advocating could by no means be regarded as strictly specific in view of the results that had been obtained by other investigators. These results have since been markedly extended and may now be more readily classified than a year ago. In the first place, it has been found that beneficial effects of a very similar nature and characterized by similar reactions to those described have been produced in typhoid fever by the administration of vaccines prepared from bacteria other than the Bacillus typhosus. Kraus[25] was the first to note that he obtained beneficial results on the intravenous injection of colon as well as of typhoid vaccines. Ludke[26] and Lucksch[27] both obtained results with colon vaccine, and the latter author obtained a favorable effect by gonococcus vaccines and by sodium nucleinate. Zupnik[16] has utilized a vaccine made from the mouse typhoid bacillus as less toxic and as useful as true typhoid vaccine. Reibmayr,[28] who has employed colon and cholera vaccines in typhoid, as well as typhoid vaccine, concludes that the former, although beneficial, are not as good as that derived from the specific microorganism.

In a similar manner it has been found that other acute infectious diseases may be beneficially affected by the intravenous injection of their corresponding vaccines or of other vaccines. Thus, Kraus[29] notes the beneficial effect produced in cases of puerperal sepsis and pyocyaneus infections by the use of a colon vaccine. Rhein[30] notes the beneficial effect in paratyphoid fever of typhoid vaccine. Nolf[31] utilized peptone solutions advantageously in streptococcus infections. Miller and Lusk[32] have employed typhoid vaccine by intravenous injection advantageously in acute, subacute and chronic arthritis.

This leads to a consideration of the less complex proteins and, indeed, simple inorganic salts that have been employed with claim to equally favorable results both in typhoid and in other acute infections. Ludke[26] has described the use of deutero-albumose in typhoid, Weichardt[33] the employment of albumin solutions, and Nolf, as already mentioned, has used one-half a gram of peptone dissolved in 200 c.c. of normal saline and administered intravenously. Baradulin[34] has utilized dextrose solutions in considerable amount in cases of surgical sepsis, and Weichardt and Engländer[35] have obtained similar results with normal physiological saline alone. The use of colloidal gold (Colibiase) by Letulle and Mage,[36] Barachon,[37] Gay,[38] Labbé and Moussaud,[39] and Delbet[40] would seem simply another instance of the nonspecificity of the reaction that we have described.

It should be reiterated that in each and all of these instances the beneficial effects produced, which are undoubted, are brought about by the *intravenous* injections of a nonspecific vaccine, a less complex protein, or even a complex or

simple organic or inorganic substance. These injections, moreover, have all been characterized by the train of symptoms we have described with typhoid vaccines, of chill, temperature excursion, and the results in the nature of critical recovery have been similar, though probably differing in relative percentage.

MECHANISM OF CURE IN INTRAVENOUS VACCINE THERAPY.

This proof of the nonspecificity of the most favorable results that can be produced by a typhoid vaccine in typhoid fever should not only not discourage us but lead us to inquire further into the mechanism of the reaction on which this benefit depends. In our previous work (Gay and Claypole, Gay and Chickering) we have been led to attribute significance to two factors, which we believe are not only concerned in, but are of importance in the reaction which is produced by intravenous vaccine injections. The first of these factors is the hyperleucocytosis which following our observations has also been found to occur by a number of authors (McWilliams,[41] Holler,[23] Löwy, Lucksch and Wilhelm,[20] Lucksch,[27] and Rohonyi[42]). It has also been shown that many of the substances which in a nonspecific manner may lead to the same effects as, for example, nucleon (Melnikowa and Wersilowa[43]), and colloidal gold (Busquet,[44] Barachon[37]), also increase the leucocyte count, to which fact their results have been attributed.

As a second factor on which the degree of benefit obtained would seem to depend is the antibody content of the patient. This we have already brought out in previous work and repeated in this article, where it is found that the strength of the Widal titer seems to vary directly with the benefit that results from injection. This observation, moreover, would agree with the incidental observation of a number of observers that prognosis in general is more or less dependent on the strength of antibodies in the patient, and, particularly, that the prognosis of vaccine injection likewise bears some relation to the degree to which the individual has reacted against the infection. Of like significance is the extremely mild and favorable course of typhoid fever in the vaccinated. Koranyi[45] has found that the opsonic index rises after vaccine injection in typhoid, and Inez Smith in our laboratory has apparently been able to demonstrate a distinct relation in the degree to which the opsonic index increases after intravenous injection and the result produced. In other words, the opsonic index rose much more markedly in the cases that were abortively cured than in either the benefited or the unaffected cases. We have frequently found that the agglutinin titer rises after the vaccine injection.

Our previous hypothesis as to the mechanism by which cure was effected in the most striking instances was, briefly, that it was due to a cooperation between the leucocytes that were called out by the injection and the antibodies already present in the patient, which latter substances acting as tropines caused the digestion and destruction of the typhoid bacilli in the body by the increased white blood corpuscles. We gave several reasons for regarding this mechanism as probably apart from the now proved relation of the antibody strength and the leucocytic increase to the result obtained. In the first place, it can be shown by blood culture in some, though by no means all, instances that the bacteria disappear rapidly from the peripheral circulation following the injection. Similar results

have been obtained by other investigators. This working hypothesis may or may not be the correct or ultimate explanation of the results produced by this nonspecific reaction in typhoid fever and in other acute infections. There are other explanations which are probably no more ultimate and which, indeed, are not of necessity in any direct conflict with the one we have presented. Jobling and Petersen[46] have attributed the results produced by the reaction as due to a disturbance of ferment, antiferment balance, the antiferment being absorbed and the ferment being allowed to act. This explanation is certainly a very attractive one. Teague and McWilliams[47] in recent experiments on rabbits also bring forth certain results which must be taken into consideration in explaining this type of therapy. They find that the injection of vaccine in rabbits produces a refractory condition which renders these animals temporarily more resistant to infection with the typhoid bacillus. They would attribute the results produced in the favorable cases of treatment to an overflowing of bactericidal substances, which are found to be present in the circulating blood, into the remoter lymph spaces which serve as metastatic foci in typhoid fever, and the consequent destruction of the bacteria that are present in them.

It makes very little difference whether we regard the leucocytes or bactericidal ferments in the serum as the cause of the ultimate destruction of the bacteria, with which recovery seems to be associated. It is probable, indeed, that the white blood cells are the sources of the ferments, and we see no reason why these various factors and experiments that have been brought out by Teague and McWilliams, Jobling and Petersen, and ourselves will not eventually stand in harmony in the ultimate explanation which may soon come.

In our previous article we expressed the hope that it might be possible to increase the percentage of most favorably reacting cases by supplying the hypothetical lack of antibodies, which seems to be characteristic of the unaffected cases. We mentioned two or three cases in which we apparently were able to produce abortive cures, although the antibody content was low, by injecting an immune serum followed by vaccine. We have since continued these experiments, but it must be confessed with no very encouraging result. In the first place, it seemed necessary to determine the best type of protective or curative antiserum that could be produced. We have utilized goats for the purpose and immunized individual animals on the one hand with sensitized, and on the other with unsensitized living typhoid polyvalent vaccines. It was found possible to compare the protective value of such sera with considerable accuracy by injecting them subcutaneously in mice with a subsequent intraperitoneal injection of typhoid bacilli grown on blood media. No striking differences in the protective value of the unsensitized as against the sensitized sera was evident. We then tried the effect of such sera in a few human cases of typhoid and found that the serum alone usually produced the same type of nonspecific reaction brought about by the vaccine. A great deal of work has recently been done in France on the use of antityphoid serum of this general type, particularly the serum advocated by Rodet. In a series of articles from 1910 to the present time Rodet and his collaborators[48] have described the preparation of an antityphoid serum in horses and the results produced by it. Similarly favorable results have also been ob-

tained by Remond and Manvielle[49] and Étienne.[50] A survey of their results, however, does not lead one to believe that the results with the serum even when given intravenously are as good as those produced by means of vaccine, and, indeed, the results produced would seem due rather to the reaction which the injection causes, and which differs only in degree from the reaction produced by vaccine, than to any antibodies that are present in the serum. Abortive cures are reported by these observers; Étienne, for example, in 12 cases out of 200, and Rodet in a somewhat larger percentage, but these abortive cures would seem to be less frequent than when vaccine is employed, and the shortening of the disease, although distinct, is by no means as pronounced as by the use of vaccine.

In view of the nonspecificity of the characteristic reaction on which the vaccine treatment of typhoid depends, it may be questioned why we continue to advocate typhoid vaccine in preference to any of the other substances that have been employed. To repeat our opinion of a year ago, we would say that typhoid vaccine alone can build up the specific immunity to the typhoid bacillus, on which, at least in part, the cure may depend, and with which a lessening of complications would seem certainly to be associated. Typhoid vaccine, moreover, when employed in the form of the Gay-Claypole sensitized sediment is a harmless substance, convenient to employ and active in very small amounts. Again, the degree of leucocytosis on which the result obtained would seem in part to depend is, we still believe, increased in a relatively specific manner when a sensitized vaccine is used. We are aware that McWilliams[51] has been unable to repeat some of our experiments on specific hyperleucocytosis. We are not prepared to state to what her failures were due.

Zinsser and Tsen[52] have found at least that immune animals react more markedly to bacterial injections than do normal ones. They do not, however, agree with us that this reaction is absolutely specific in that heterologous antigens will apparently produce the same effect. It is unfortunate that neither of these investigators has repeated our experiments, in which we found that normal animals reacted more markedly to sensitized vaccines than to untreated vaccines.

SUMMARY AND CONCLUSIONS.

This article records our experience in the treatment of ninety-eight authenticated cases of typhoid fever over a period of two and one-half years by the intravenous injection of a polyvalent sensitized typhoid vaccine sediment (Gay-Claypole vaccine). The cases were observed for the most part in hospitals, but to some extent in private houses. The series includes cases studied in New York City, as well as in California. We regard the treatment as indicated in any case of typhoid fever that is still febrile, on the basis of our results which seem excellent. The mortality in these cases was low (6.6 per cent) and the complications few. Relapses were distinctly reduced in those cases in which the intravenous injections were followed by a series of three subcutaneous inoculations after the temperature had reached normal.

Our estimate of the beneficial results which have followed this treatment, apart from the probable reduction of mortality and complications, is based on the following facts:

1. In one-third of the cases the temperature fell critically following the first or second injection, and a permanent normal was established on the average within a week after beginning treatment.

2. In a second series of cases each injection was followed by a permanent decrease of a degree or more in the temperature.

3. Subjective benefit, apart from temperature changes, seemed to follow the majority of inoculations.

The treatment is perfectly harmless with the particular vaccine and under the conditions employed.

A detailed consideration of similar methods of therapy that have been studied with increasing interest in the last two years would seem to show that intravenous injections are far superior to subcutaneous injections of vaccine, and that sensitized vaccines are better than plain typhoid vaccines. It appears that the reaction is not strictly specific in that a similar type of reaction, chill, temperature excursion, etc., which is characteristic of the intravenous vaccine injections, may be produced by similar administration of other vaccines, of other proteins, and, indeed, of salt solution. There is some evidence, however, that relatively better results are produced by the use of the typhoid vaccine which in addition aids in establishing an active immunity against the specific micro-organism concerned. The exact mechanism by which the benefit or cure is effected is as yet uncertain, but it is at least marked by a temporary increase in the number of leucocytes and is probably dependent on the strength of antibodies already present in the individual patient. Our efforts to supply these antibodies by means of an antityphoid serum have not met with great success, and the beneficial effects that others have obtained with antityphoid serum would seem to be due to a mechanism similar to that following vaccine injections, but less effective than the latter.

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THE RELATION OF PHYSICAL CHEMISTRY TO THE IRRIGATION OF WOUNDS*

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So many factors enter into the healing of a wound that it is very difficult to make an analysis of the response to treatment in any moderate number of cases. It seems permissible, therefore, to discuss certain physiological principles which should be kept in mind in any investigation of the methods of treatment. If a wound can be sterilized, it may be closed, and hence is outside the present discussion. Since the object of the irrigation of wounds is the removal of bacterial toxins and necrotic tissue as long as the wound remains infected, the irrigation must be continued for a long time. The prolonged diffusion between the irrigating fluid and underlying tissues necessitates unusually detailed precautions for the protection of these tissues. Although the ultimate sterilization of the wound is the object of the treatment, no drug has been found that will kill all classes of bacteria embedded in tissue without injuring the tissue, and hence time must be allowed for the development of antibodies. For this reason the antiseptic properties of the irrigating fluid must be subordinated to its physiological properties.

During the past ten years, I have investigated the effects of various media on many classes of cells. Not all of this work has been published, but the main points, as well as references to the literature and special methods of work may be found in my book.[1] My early work was done on bacteria, moulds, protozoa, egg cells and embryos. For instruction in the preparation of tissue cultures of warm-blooded animals, I am indebted to Montrose Burrows, with whom I had the good fortune to be associated for several years.

Conditions which impair the functions or kill cells may be roughly divided into radiations, changes in temperature, and mechanical, osmotic, and chemical changes, the last including the effects of the electric current. Some cells are

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more resistant than others, and their relative resistance to different classes of adverse conditions varies greatly. Thus some cells are unusually resistant to osmotic changes and quite sensitive to chemical changes. As a rule a cell that is sensitive to one chemical change is proportionately sensitive to another, the few exceptions being in relation to specific drugs. The ratio of the concentration of one substance to another may have to be considered in the case of those having antagonistic actions.

Electrolytes are dissociated into ions, and it is usually the electro-positive ions that are most toxic, the order being about as follows, commencing with the least toxic: Na, Cs, Li, Rb, K, Mg, Ca, Sr, Ba, Al, Mn, Sn, Fe, Tl, Co, Ni, Zn, Pb, Cd, As, Cu, Bi, Sb, Hg, Ag, Pl, Pt, Au. H and OH ions kill most rapidly owing to their great speed of diffusion, but when the time element is not considered, they must exceed certain concentrations in order to be toxic and are difficult to place in the series. This question will be considered below in connection with special methods for the estimation of the concentration of H and OH ions. To facilitate the slow administration of an ion it may be combined in an organic compound which slowly liberates it, as As in salvarsan and cacodylic acid, Ag in argyrol, and Hg in $K_2Hg(CN)_4$. Na, K and OH ions antagonize the action of Ca, Mg, and H ions. The antagonism between H and OH is complete because they combine to form H_2O. The antagonism between Na and Ca is almost complete but it requires but a little Ca to antagonize a large amount of Na. The other antagonisms, and many not mentioned, are less complete. The alkali-metals are similar to one another in action, but only Na and K have been thoroughly studied. As a rule K is more toxic to human cells, but Na is more toxic to many cells of lower animals and plants. The antagonism between $HgCl_2$ and NaCl is due to the formation of the poorly dissociated Na_2HgCl_4, and holds only where the exposure is of short duration.

The toxicity of the indifferent anesthetics is proportional to their effect in lowering the surface tension of water, and hence the toxicity of their solutions is proportional to the number of drops that fall from the end of a pipette after one filling. The dropping surface of the pipette should be about 5 mm. in diameter, and the delivery tube constricted so that at least a minute is required for all of the fluid to drop out. Not only the substances used for anesthesia, but a larger number of substances including ethers, alcohols, ketones, esters, soaps, nitriles, halogen hydrocarbons, benzol derivatives, alkaloids and many other substances, fall in this class. Some of these substances which are not sufficiently soluble for antiseptics may be beneficial to wounds, as for example, iodoform, which increases the activity of phagocytes.

Chemically active substances, such as phenol and cresol, and especially those with powerful oxidizing or reducing action, may also lower the surface tension of water; but are far more toxic than would be expected from the surface tension of their solutions. Formaldehyde does not owe its toxicity entirely to its reducing action, but forms additive compounds with proteins, which may decompose again with the liberation of formaldehyde. Oxidizing substances are, however, reduced by cells, and an ideal local antiseptic would be one whose reduced product is indifferent. Hydrogen peroxide falls in this class,

but is not a powerful oxidizing agent and is decomposed by catalase so rapidly as to render the large percentage of it ineffective. It acts chiefly as a mechanical cleanser. If infusoria are placed in a solution of H_2O_2, the latter penetrates their protoplasm and is decomposed on the inside with the liberation of bubbles of oxygen which burst and destroy the cells. More useful oxidizing agents are iodine and chlorine, especially the latter since the HCl formed on its reduction may be neutralized by $NaHCO_3$ that has been added and thus rendered indifferent. According to Dakin and his collaborators, chlorine forms chloramines when it acts on protoplasm, and these chloramines have an antiseptic action. It is true, however, that chlorine oxidizes many organic compounds, with the liberation of HCl. Chlorine gas escapes rapidly from its solution in water, but this may be retarded by the addition of a base, transforming it into hypochlorite. Its oxidizing power is impaired, however, if the reaction is very alkaline, but may be restored by bubbling CO_2 through the solution.

It makes little difference whether chlorine gas (Cl_2) or calcium hypochlorite (CaO_2Cl_2) or sodium hypochlorite (NaOCl) or hypochlorous acid (HOCl) are used in so far as their antiseptic action is concerned (except that the solution should not be strongly alkaline), as they are readily and spontaneously converted into one another according to the formulæ:

$$Cl_2 + 2NaOH = NaOCl + NaCl + H_2O$$
$$2Cl_2 + 2Ca(OH)_2 = Ca(OCl)_2 + CaCl_2 + 2H_2O$$
$$Cl_2 + H_2O = HOCl + HCl$$

Since solutions of all these substances give off chlorine to the air and do not retain their original concentration, some rapid method of determining their concentration is useful. The most accurate method is titration with arsenious acid, since it is not affected by chlorate, and many refinements in iodimetric standardization may be used, but the following simple method suffices for the present purpose.

The process depends on the liberation of iodine by the chlorine and the titration of the iodine with sodium thiosulfate ($Na_2S_2O_3$) commonly called "hypo" by photographers, the reaction being:

$$Cl_2 + 2KI = I_2 + 2KCl \text{ or}$$
$$NaOCl + 2KI + 2HCl = I_2 + NaCl + 2KCl + H_2O \text{ and}$$
$$2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$$

If specially pure sodium thiosulfate crystals, showing no efflorescence can not be obtained, a saturated solution in distilled water at body temperature is made and cooled until crystals form. These crystals are dried in air, and have the composition $Na_2S_2O_3.5H_2O$. Of these crystals 24.832 grams are weighed and dissolved in distilled water that has been boiled to remove the CO_2, so as to make a liter of tenth normal solution. This solution must be protected from the CO_2 of the air by means of soda-lime tubes, and is therefore more conveniently handled in an automatic burette. In making the titration, a few crystals of KI and about a cubic centimeter of HCl are placed in a flask and 10 c.c. of the chlorine

solution run in from a pipette, then titrated with the thiosulfate solution until the iodine color disappears. Dakin and Carrell state that the proper strength for wounds is .45 per cent to .5 per cent, but this is more conveniently stated as 0.127 to 0.141 normal, as it requires 12.7 to 14.1 c.c. of the thiosulfate to titrate 10 c.c. of the irrigating fluid that is of proper strength.

Apart from the chlorine or other antiseptic in the irrigating fluid, it should be physiologically normal. Suppose the chlorine were dissolved in distilled water, for example. The chlorine would be reduced or bound on meeting the first layers of tissue or exudate, but the water would penetrate deeper and injure the tissue without affecting the bacteria that had escaped the chlorine. If the chlorine were dissolved in an isotonic solution of NaCl, this salt in pure solution would be injurious and the HCl formed on reduction of the chlorine still more toxic, although both would be somewhat lessened in action by the admixture of exudate. In a hypertonic solution the exudate would be increased, but the hypertonicity is injurious to tissues.

Dakin's fluid is usually prepared from a solution of calcium hypochlorite by precipitating the lime with a mixture of carbonate and bicarbonate of sodium. Although $CaCO_3$ is only 0.0013 per cent soluble in distilled water, Dakin's fluid usually contains more than this, and there is probably always enough to antagonize the toxicity of the Na. It is necessary, however, to add more than the physiological concentration of Na in order to precipitate the greater part of the Ca, and the solution is hypertonic. The freezing point of human blood is —0.56° C., but the samples of Dakin's fluid that I have tested froze at —0.7 to —0.9°, and were therefore very hypertonic. Even after precipitating a large part of the lime by bubbling CO_2 through the solution, it was necessary to add so much $NaHCO_3$ to precipitate the remainder of the lime that the solution was hypertonic. The precipitation depends on the alkalinity and the concentration of CO_2 and other substances. Oxalic acid can not be used to precipitate the lime as the chlorine immediately oxidizes it to CO_2 and the antiseptic action of the fluid is simultaneously lost. I have made isotonic irrigating fluids by running chlorine gas into physiological salt solutions in which the NaCl had been replaced by a chemically equivalent amount of $NaHCO_3$. The physiological properties of the salts will now be considered.

The physiological salt solutions in common use in laboratories are by no means perfect, as illustrated by the following comparison of their percentage composition with that of the diffusible salts in blood serum:

	NACl	KCl	$CaCl_2$	$MgCl_2$	$MgSO_4$	$NaHCO_3$	NaH_2PO_4
Ringer-Locke	.9	.042	.024	—	—	.01-.03	—
Tyrode	.8	.02	.02	.01	—	.1	.005
Serum	.65	.04	.02	—	.012	.33	.013

In the data for serum, only the diffusible salts are included, and owing to ionization and recombination, a larger number of salts are present than indicated in the table. The expression of the data in this form is for comparison with physiological salt solutions. Tyrode has been the only previous investigator to introduce Mg into physiological salt solution and apparently no one but the writer has used SO_4. The formula for physiological salt solution successfully used by the writer is that given in the last line in the above table, and more

nearly imitates serum. Only one difficulty might arise. The blood is under a partial pressure of CO_2 of 40 millimeters of mercury, or 6 per cent of an atmosphere, and when this pressure is relieved it becomes more alkaline, thus initiating coagulation and other changes. A solution of the blood ash would become alkaline in the same manner, and hence toxic to living cells with which it came in contact. A solution of diffusible serum salts should be tightly stoppered to prevent the loss of CO_2, or the CO_2 thus lost should be replaced by bubbling the gas through the solution. The proper reaction may be reached by bubbling the alveolar air of a normal man through the solution. If pure CO_2 is bubbled through the solution, some means must be used to ascertain when the proper reaction is reached. The most convenient way is to determine the hydrogen ion concentration, as given in the following paragraphs.

It is more convenient to express alkalinity (or acidity) in terms of the minus logarithm of the hydrogen ion concentration, abbreviated to PH. The PH of a neutral solution is about 7, whereas that of blood is 7.5, and that of sea water about 8. The extreme limits of the variation of the PH that is compatible with the life of most cells is from 5 to 9, and the majority of cells do not bear variations beyond 6 to 8. The writer made some experiments with species of mould sealed in glass tubes by fusing the glass so that no fresh spores could enter. The mould thrived in a saturated solution of boric acid containing 3 per cent pure NaCl; but if enough NaOH was added to bring the PH up to 8, no more growth took place (alkaline limit). Clark and Lubs[2] give the acid limits, of moulds and bacteria at 2.3—6, and alkaline limit 9.4.

The PH of any solution, free from NH_3, H_2S, Cl_2 and certain antiseptics, may be determined by means of the hydrogen electrode, and the PH of many solutions may be determined approximately by means of indicators and solutions standardized by means of the hydrogen electrode. For solutions containing chlorine (Dakin's fluid) none of the ordinary methods may be used. The chlorine poisons the hydrogen electrode and destroys the indicators with extreme rapidity. The method used by the writer consists in selecting a series of indicators in aqueous solution and noting the color of the indicator solution the moment it is dropped into a large quantity of Dakin's fluid. The indicator solution must be brought to the yellow color before being used. If the resulting colors are recorded and compared with the following table, the PH may be determined approximately.

	3	4	5	6	7	8	9	10
Tetrabromphenolsulfonphthalein	yellow	bluish	blue	blue	blue	blue	blue	blue
Methyl red	red	red	orange	yellow	yellow	yellow	yellow	yellow
Dibrom-o-cresolsulfonphthalein	yellow	yellow	yellow	purplish	purple	purple	purple	purple
Dibromthymolsulfonphthalein	yellow	yellow	yellow	yellow	green	blue	blue	blue
Neutral red	red	red	red	red	orange	orange	orange	orange
Phenolsulfonphthalein	yellow	yellow	yellow	yellow	red	orange	red	red
o-Cresolsulfonphthalein	yellow	yellow	yellow	yellow	yellow	orange	red	red
a-Naphtholsulfonphthalein	yellow	yellow	yellow	yellow	yellow	green	blue	blue
a-Naphtholphthalein	white	white	white	white	white	greenish	green	green
Thymolsulfonphthalein	yellow	yellow	yellow	yellow	yellow	green	green	blue
Phenolphthalein	white	white	white	white	white	white	pink	red
Thymolphthalein	white	white	white	white	white	white	white	blue

Most of these indicators are more soluble in alcohol, but alcoholic solutions must not be used, owing to formation of HCl. Phenolsulfonphthalein and a-naptholsulfonphthalein which will act only in aqueous solution and then very poorly, should be avoided if possible. These indicators are quickly attacked by

the chlorine and changed into yellow compounds which may lead to erroneous interpretation of the results unless careful observation is assured. Obviously not all of the indicators are necessary, and a choice may be made among those of the same range. If a physiological fluid is made of neutral salts, it can be brought to a PH of about 7.5 by the addition of 0.0035 per cent $NaHCO_3$ and blowing air through until equilibrium is attained, after which it will not change on exposure to air, but has little buffer action against acid, and alkalies. If CO_2—free fluids are used, the PH may be adjusted by the addition of borate or phosphate buffer mixtures, but these will absorb CO_2 slowly from the air and become less alkaline.

In case of solutions into which chlorine gas is to be run, practically all of the salt content should be bicarbonate, as the base is necessary for the formation of the hypochlorite and the CO_2 for the reduction of alkalinity. The solution which I have used chiefly contains 1.25% $NaHCO_3$, 0.04% KCl, 0.02% $CaCl_2$, 0.025% $MgSO_4 \cdot 7H_2O$, and 0.01% NaH_2PO_4.* A brown glass bottle is filled with this solution and inverted in a basin of the solution, and chlorine gas is passed into the bottle through a glass tube until it displaces more than half of the solution. The tube is removed and the stopper inserted and the bottle shaken so that the solution will absorb the gas, the stopper being lifted occasionally so as to admit air in the space from which the gas has been absorbed. The solution is then titrated and if too strong, diluted with some of the gas-free solution.

If a cylinder of chlorine gas is not at hand, the gas may be generated as follows: A flask is about one quarter filled with powdered manganese dioxide and HCl added until well moistened. A perforated rubber stopper is inserted in the flask and connected with a bent glass tube to lead the gas to the bottom of the inverted bottle. When the gas ceases flowing it may be started again by immersing the flask in water and heating it to boiling. Still more gas may be obtained by the addition of more HCl. If a separatory funnel is at hand it may be used to introduce more HCl through a second perforation in the rubber stopper without lifting the stopper, but it is a matter of convenience and not necessary. As the chlorine bubbles out of the concentrated hydrochloric acid in the flask, some HCl gas is carried off with it, but this quantity is negligible if the first charge of the acid is entirely soaked up in the powdered MnO_2 and no heat is applied at the start.

The PH of the irrigating fluid may be determined as indicated, and lowered by bubbling CO_2 through, or raised by bubbling air through it. The PH should not be allowed to exceed the limits 5 to 7.5 unless it is desired to irritate the tissue. The PH depends on the ratio between CO_2, which tends to acidify the fluid, and NaOCl, which has an alkaline reaction due to hydrolysis according to the equation.

$$NaOCl + NaCl + H_2O = 2NaOH + Cl_2$$

When an organic compound such as oxalic acid, for instance, is added to the Dakin's fluid, this alkaline hydrolysis is prevented, owing to the reaction:

$$\begin{matrix} COOH \\ COOH \end{matrix} + NaOCl = 2CO_2 + H_2O$$

*This solution may be charged with chlorine by means of a sealed tube of chlorine now on the market.

According to Dakin,[3] when hypochlorite comes in contact with proteins the following reaction takes place:

$$>NH + NaOCl = >NCl + NaOH$$

and hence the solution would become more alkaline. In a mixture of proteins and other organic substances, reactions of both types may occur and tend to balance one another, neither increasing nor decreasing the alkalinity to a marked degree.

It should be noted that in both types of action of NaOCl on organic substances (in which oxygen or chlorine combines with the organic substance) the organic substance is oxidized. Oxidation, in the broad sense, is not combination with oxygen but an increase in the positive valences or decrease in the negative valences. For example, in the oxidation of HCl by manganese dioxide to form chlorine gas as described above, the electro-negative chlorine ions lose their negative charges (free valences) and become electro-neutral chlorine atoms as expressed by the equation:

$$2Cl' + O + 2H^{\cdot} = Cl_2 + H_2O$$

in which the negative charges of the anions are express by (′) and the positive charges of the cations by (˙) and show that the negative charges of $2Cl'$ are transferred to O, thus enabling it to combine with $2H^{\cdot}$.

According to Dakin, it is not the NaCl which sterilized the wound but chloramines formed by its action on organic substances, and he proposes to substitute a chloramine ($CH_3 \cdot C_6H_4 \cdot SO_2Na : NCl$) for the NaOCl. He states that chloramine does not give off chlorine in solution (and hence it has not the oxidizing power of NaOCl). This is in harmony with Dakin's statement that chloramine is not as effective as NaOCl in removing necrotic tissue. Dakin, Cohen and Kenyon[4] observed that guinea pigs would tolerate a subcutaneous injection of 1 gram of chloramine per kilo. From this it may be concluded that the oxidation products formed by the action of NaOCl on necrotic tissue are not unusually toxic if absorbed, and hence the advantage of NaOCl over phenol is obvious.

In order to prevent the absorption of bacterial toxins from the wound, Wright advocates the use of very hypertonic (5%) NaCl containing oxalate. It should be remembered, however, that this solution is toxic to adjacent tissues, and therefore it seems advisable to try other methods. If the flow of Dakin's fluid, for instance, is rapid enough, it should carry away these toxins to a large extent. Isotonic Dakin's fluid would be absorbed by the blood especially if there was a lack of sufficient water in the body. It has been shown by Starling and especially by F. H. Scott[5] that it is the osmotic pressure of proteins that enables the blood to absorb isotonic solutions, since the blood vessels are less permeable to proteins than to other substances. Only when the blood pressure in the capillaries equals the excess of osmotic pressure does absorption fail to occur. It has been shown by Hertzler, Murphy and others that absorption from the peritoneum can be prevented by the administration of large quantities of water to the patient, and this method might be used to prevent the absorption of an isotonic irrigation fluid from a wound. A continuous rectal irrigation with water might be used simultaneously with the irrigation of the wound with isotonic Dakin's fluid.

NaOCl as such has little oxidizing power, but its dissociation products are powerful oxidizing agents. Increase in the alkalinity of the solution decreases the dissociation of NaOCl and hence decreases its oxidizing power. The dissociation products are used up when the fluid comes in contact with tissue, thus leading to dissociation of more NaOCl and decrease in the concentration of the solution, finally to zero. It is obvious that rapid irrigation with a dilute solution will have the same oxidizing effect as slow irrigation with a more concentrated solution, and have the further advantage of more thorough washing out of the wound. This is true of the wound cavity, but when thick masses of necrotic tissue are acted on, the diffusion into them of NaOCl is regulated by its actual concentration on the outside, or in other words, penetration depends on concentration. Since, however, it is not desired that the NaOCl penetrate the living tissue, lower concentrations are preferable.

The writer has prepared low concentrations of NaOCl by a simplified method, as follows: Ringer's fluid is placed in a glass jar, and 2 electric light carbons inserted. A direct electric circuit is connected with the carbons and the current passed for about 15 minutes, when the NaOCl is titrated. The current need not be more than a few volts, and if the lighting circuit of 110 or 220 volts is used, it must be passed through a resistance so as to prevent overheating of the solution. Not all of the NaCl can be transformed into NaOCl. CO_2 is passed through the solution until the required PH is reached. Heitz-Boyer has shown that such a solution can be conveniently made on board a hospital ship by the electrolysis of sea water. The writer has for many years (in case of emergency) used sea water diluted with 2 or 2.5 volumes of fresh or distilled water in place of Ringer's fluid. This solution contains an excess of magnesium but not sufficient to be toxic to tissues. The PH is about 8 and hence some CO_2 should be added. Undiluted sea water is very irritating to wounds and delays healing, especially if some evaporation occurs. If the sea water is washed out of a wound, it heals more quickly even without the application of antiseptics. This does not favor the hypothesis that the toxic action of sea water on a wound is due to pathogenic bacteria in the sea water; it seems more probably due to its salts.

Dakin's fluid, made with the physiological precautions discussed in this paper, has been tried on large wounds in dogs and men. Not sufficient data have been collected to show that these precautions are always worth the trouble, but on the other hand, no disadvantages of such a solution have been noted. The theoretical considerations that led to the experiments recorded in this paper are given in the following discussion:

DISCUSSION.

Cell media or solutions coming in contact with living cells are of two physiological classes: nutritive and protective, as shown by the work of Sydney Ringer, J. Loeb, Osterhout, and many others. A great deal of speculation has arisen over the protective action of cell media and especially over the antagonistic or antitoxic action of ions on one another as evidenced by their combined effect on cells. The writer was the first to show by chemical analysis that the toxic action of ions and other substances on animal cells is associated with changes in permeability, and to quantitatively measure the diffusion of determined substances

from the cells on increase in permeability. Thus a quantitative and qualitative method of studying permeability and the toxic action of substances in the cell medium and the antitoxic action of other substances was built up. It had been shown long ago that increased diffusion of substances out of cells occurs on their death, and permeability had been supposed to be a means of regulating physiological activity. The writer has shown that the permeability of muscle increases on contraction and the permeability of eggs increases on fertilization, and if increase in permeability is not brought about by means of the sperm or other agent, no segmentation of the egg occurs. Fertilization of the frog's egg is associated with the outward diffusion of Na, K, Mg, Ca, Cl, SO_4, and CO_3 (in addition to the CO_2 or other products of metabolism). A greater than normal increase in permeability is associated with pathological changes or death. The permeability of certain fish eggs may be increased by certain alkaloids such as strychnine, by caffeine and by pure solutions of salts. If these eggs are placed in a toxic solution of quinine, for instance, the rate at which Na, K, Mg, Ca, Cl, and SO_4 diffuse out of them is associated with their pathological condition. A certain increase in permeability is associated with the development of the eggs into one-eyed embryos or embryos with various defects in the nervous or circulatory systems. These abnormal embryos may hatch, but maximal increase in permeability is associated with death.

Physiological permeability is reversible, pathological permeability is not instantaneously reversible, and permeability associated with death is irreversible. Stimulation of cells consists in reversible increase in permeability. In general, methods used to kill cells, if carefully applied, may be used to stimulate them; i. e., to cause muscle to contract, or to cause eggs to develop without the necessity of the male element. This increase in permeability is associated with the very unstable surface layer of protoplasm. This layer may be compared with a copper ferrocyanide precipitation membrane. The latter will not allow sugar or $MgSO_4$ to pass through it, whereas water passes freely; but if NaCl is added to the water, sugar will pass through the membrane. In other words, NaCl increases the permeability of the copper ferrocyanide membrane. Protoplasm is made up largely of colloids, and it seems probable that in its normal state the colloid particles on its surface are so close together that diffusion is impeded. Some cells do not allow water to diffuse in or out, while most cells are freely permeable to water and oxygen. The permeability to other substances varies with the cells and their physiological states. Anything that causes a rearrangement of the colloid particles of the cell surface alters its permeability. The electric current has this effect and is the most rapid means of stimulating or killing cells. Cells may be sensitized so that they are more easily stimulated or killed. This sensitization may consist in a subminimal increase in permeability so that a smaller increase than normally necessary will result in stimulation, or in some other change increasing the instability of the colloids. Anesthetics, when applied in certain definite concentrations, dissolve in certain of the colloid particles, thus increasing their size, or surround them with a protecting layer, thus increasing the difficulty of stimulation; but when the concentration of the anesthetic reaches the lethal dose for that cell, some of the colloid particles are dissolved out of the

surface layer by the anesthetic or changed in some other way so that the permeability is increased and death results. A certain degree of impermeability is necessary in order to preserve the integrity of the cell, as otherwise its soluble constituents would be lost, but certain special degrees of semipermeability are associated with the metabolism and functions of the cell, and facilitate the escape of waste products and the absorption of necessary substances.

Since the surface protoplasm of cells varies, their sensitivity to toxic substances varies. Pascucci showed that those red blood corpuscles containing the larger amount of lecithin are more easily rendered permeable (laked) by soaps and other substances which attack lecithin, whereas the reverse is true of those erythrocytes containing the larger amount of cholesterin. No doubt the protein constituent of the surface of the corpuscle affects the specificity of laking agents for blood of different species of animals, but even less seems to be known of the constitution of proteins than of lecithin and cholesterin. This form of specificity has not been sufficiently worked out to be applicable to many groups of pathogenic organisms. More success has been achieved in finding specific poisons for protozoa than for bacteria, and in general, bacteria seem to be more resistant than are tissue cells.

Permeability studies have given the clue to the antagonistic action of salts (ions) on one another. Any ionic species, when in sufficient concentration and unaccompanied by antagonistic ions, increases the permeability to such an extent that death occurs. Tissues of mammals seem to bear higher concentrations of Na ions than of any others, and tissues of marine animals seem not to be killed by great changes in concentration of Mg ions; but either of these, when maintained in absolutely pure solution, is deadly. $Na^{\cdot}$ causes increase in permeability, and $Mg^{\cdot\cdot}$ causes decrease in permeability; and hence they are antagonistic to one another when applied in the proper ratio, but a pure solution of $Mg^{\cdot\cdot}$ of sufficient concentration causes increase in permeability. Within physiological limits, OH' causes increase in permeability and $H^{\cdot}$ causes decrease in permeability, but a high concentration of $H^{\cdot}$ causes increase in permeability. Likewise it is possible under certain conditions to show that $Ca^{\cdot\cdot}$ causes decrease in permeability but the quantitative side of this question needs further investigation.

The ideal irrigation fluid for wounds seems to be one which will maintain the normal permeability of the tissue cells and at the same time wash out and sterilize the cavity of the wound and sterilize the surface of its walls, the antiseptic being one that will be used up and thus prevented from penetrating the living tissue. Since many proteins are soluble in salt solutions, necrotic tissue should break up if the formation of bacterial zooglea are prevented. The greater the alkalinity (the farther from the isoelectric point of the proteins) the greater the solvent action, but the more irritating to the issue.

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ANTIDOTES IN MERCURIC CHLORIDE POISONING*

Second Communication

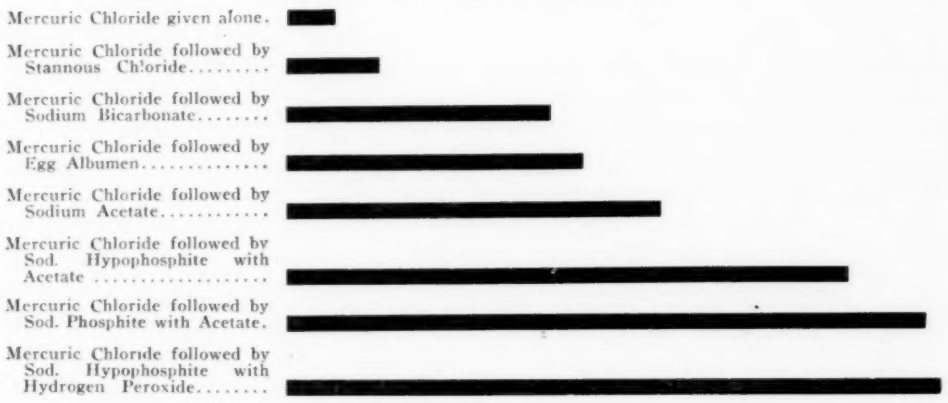
THE VALUE OF PHOSPHITE AND HYPOPHOSPHITE COMBINATIONS

By Bernard Fantus, M.D., and Emry G. Hyatt, Chicago, Ill.

In a previous study,[1] it was found that a mixture of sodium phosphite and sodium acetate (Carter's Antidote) and one of sodium hypophosphite and hydrogen peroxide gave the best results among all the antidotes experimented with. Inasmuch as the results with the antidotes named were approximately equal, as may be seen from Table I, reproduced here from the previous publication, it seemed of importance to determine which one of the two was the best for practical purposes.

Table I.

SUMMARY OF AVERAGE LETHAL PERIODS.


Mercuric Chloride given alone.
Mercuric Chloride followed by Stannous Chloride.....
Mercuric Chloride followed by Sodium Bicarbonate.....
Mercuric Chloride followed by Egg Albumen.....
Mercuric Chloride followed by Sodium Acetate.....
Mercuric Chloride followed by Sod. Hypophosphite with Acetate .....
Mercuric Chloride followed by Sod. Phosphite with Acetate.
Mercuric Chloride followed by Sod. Hypophosphite with Hydrogen Peroxide.....

CONTROL TESTS.

Owing to the importance of rigid control, one or more animals in each series were given the poison without antidote. The results are shown in Table II. We have now administered the dose of 0.04 gm. per kg. to 22 (oats and carrots fed) rabbits with a fatal result in all cases, and an average fatal period of seven and three-fourths days. All of these animals were given as much oats and carrots as they could eat. This is a factor of importance, for we have found that animals fed on carrots only were very much more resistant to mercuric chloride poisoning than were those fed on carrots and oats. We are at present engaged in determining the reason for this interesting fact, and hope to be able to report in the near future our work in this direction. We feel confident, as a result of our

*From the Pharmacologic Laboratory of the College of Medicine, University of Illinois.

observations, that 0.04 gm. per kg. constitutes a uniformly fatal dose for rabbits, provided the rabbit has been fed on oats and carrots and provided the mercuric chloride solution has been freshly prepared with distilled water; and believe that the results would have been more uniform had a weighed amount of carrots been fed previously, instead of giving the animals all the carrots they would eat in addition to oats.

In the work previously reported, the antidote was given either mixed with or immediately after the administration of the poison. This was, of course, giving the antidote an unfair advantage. For in practice such prompt use of antidote is an impossibility. We, therefore, in the work here to be reported, permitted a uniform interval of 5 minutes to elapse between the administration of the poison and of the antidote, having withdrawn the stomach tube during the interval. It seems that an antidote that displays undoubted efficiency on such a test should be accorded a place in practical therapeutics.

CARTER'S ANTIDOTE.

This mixture of sodium phosphite, 10 parts, and of sodium acetate, 6.6 parts, was advocated by Thomas A. Carter[2] on the basis of test tube experiments and of observation of its value in cases of poisoning in the human. Owing to the difficulty of being absolutely certain when life has really been saved in the ordinary conditions of medical practice, owing to the impossibility of accurately comparing the value of different antidotes, and the great practical importance of knowing which antidote is the best, the method employed in our work seems to offer an opportunity for formulating a definite opinion on these questions. By comparing Tables II and III, it will be seen that Carter's Antidote is undoubtedly of value in saving lives of rabbits poisoned with a fatal dose of corrosive mercuric chloride. While no animal in the control series survived 100 days, 7 among 21 rabbits, or 33⅓ per cent, of those treated with Carter's Antidote recovered. The average lethal period in the Carter's Antidote series was 44½ days, against 7¾ days in the control series. This result agrees very well with that obtained in our previous study of this antidote. However, even these figures probably do not represent the real value of the antidote; for, as will be seen from Table III, nearly all the animals that died succumbed within the average fatal period of the control series, as though they had not been given antidote. This suggests that, in these cases, the antidote might not have had a chance to act upon the poison, which might have been deposited in a different part of the food-filled rabbit's stomach from that in which the antidote happened to be introduced. The fact that about half of the number of animals died as though they had had no antidote, while the other half of the series recovered or showed well marked delay in the fatal period suggests that the chances are about even that antidote and poison may mingle with or miss each other in the rabbit's stomach. In a stomach that is empty, like the human stomach after lavage, the chances for antidotal action ought to be decidedly better than in the ever-full rabbit's stomach.

Inasmuch as calomel is formed, when sodium phosphite is mixed with a solution of mercuric chloride, one might expect that sodium phosphite alone would be sufficient for the purpose. This it does not seem to be, as was indicated by the results of our previous study (Table IV).

TABLE II.

CONTROL TESTS.

ANIMALS FED ON CARROTS AND OATS. DOSE: 0.04 GM. PER KG. CORROSIVE MERCURIC CHLORIDE BY STOMACH.

Rabbit	Weight	$HgCl_2$ 1%	Lived	Necropsy Lesions	
				Local	General
1	1325	5.3	3	Marked	Marked
2	1448	5.8	6	Slight	Slight
3	1325	5.3	5	Marked	Marked
20	910	3.6	3	"	"
21	905	3.6	2	"	"
22	900	3.6	2	"	"
23	1140	4.5	3	"	"
24	1225	4.9	3	"	"
25	1500	6.0	5	Slight	"
26	1355	5.4	3	"	"
44	1075	5.0	60	Necropsy Lost	
45	1625	6.5	7	Marked	Marked
82	2500	10.0	35	Slight	Slight
83	1400	5.6	4	Marked	Marked
101	1280	5.1	2	"	"
102	1392	5.6	4	"	"
103	1422	5.7	6	"	"
104	1100	4.4	4	"	"
105	1469	5.9	3	"	"
106	1379	5.5	4	"	"
107	1471	5.9	4	"	"
108	1700	6.8	2	"	"

This table shows that the average lethal period for 0.04 gm. of $HgCl_2$ per kg. is 7¾ days. No animal survived 100 days.

TABLE III.

LETHAL DOSE OF MERCURIC CHLORIDE (0.04 GM. PER KG.) FOLLOWED 5 MINUTES LATER BY 10% SOL. OF SODIUM PHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (6.6 TIMES AS MUCH). (CARTER'S ANTIDOTE.)

Rabbit	Weight	$HgCl_2$ 1%	Carter's Antidote	Lived	Necropsy Lesions	
					Local	General
4	1465	5.9	5.9	3 days	Marked	Marked
5	1632	6.6	6.6	69 "	Slight	Slight
6	1113	4.4	4.4	7 "	"	"
16	1620	6.4	6.4	100 "		
17	1485	3.9	3.9	100 "		
18	2135	8.5	8.5	12 days	Marked	Marked
58	787	3.1	3.1	4 "	"	"
59	1459	5.8	5.8	5 "	"	"
60	1057	4.2	4.2	100 "		
77	1404	7.6	7.6	100 "		
79	2024	8.0	8.0	100 "		
84	1430	5.7	5.7	100 "		
85	1220	4.8	4.8	40 "		
86	1626	6.5	6.5	100 "		
87	1580	6.3	6.3	2 "	Marked	Marked
88	1471	5.9	5.9	3 "	"	"
89	1411	5.6	5.6	6 "	"	"
92	1351	5.4	5.4	15 "	"	"
96	1675	6.7	6.7	67 "	Negative	Negative
99	1590	6.4	6.4	4 "	Marked	Marked
100	1920	7.7	7.7	3 "	Marked	Marked

Carter's Antidote, when freshly prepared in solution, has a decided antidotal value. Average lethal period 44½ days. Seven animals among 21 (33⅓%) survived 100 days.

TABLE IV.

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE.

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Lived	Necropsy Lesions	
						Local	General
H	1018	0.068	w	0.68	4 days	0	Marked
H 1	780	0.030	w	0.30	4 "	0	"
HL1	1188	0.047	f	0.47	4 "	Marked	"

Sodium phosphite given alone is of little value as antidote, av. survival 4 days.

Carter believed that the reason for the value of the acetate was to be found in its diuretic action. This can not be the case: for, in the first place, the dose is too small to have much diuretic action; and then, as we have shown in our previous study (Table V), a large excess of acetate, which ought to have a still

TABLE V.

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (100 TIMES AS MUCH).

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
H 4	1749	0.070	w	0.70	7.00	1 day	Slight	Slight
H 6	974	0.039	w	0.39	3.90	3 days	"	"
HL4	1215	0.049	f	0.49	4.90	3 "	Marked	Marked
HL6	1000	0.040	f	0.40	4.00	4 "	Slight	"

Sodium phosphite with large excess of acetate is of no antidotal value.

TABLE VI.

USE OF OLD ANTIDOTAL SOLUTIONS.

Lethal dose of mercuric chloride (0.04 Gm. per Kg.), followed 5 minutes later by antidotal solutions that were kept for varying periods of time. Numbers 31 and 32 were given antidotal solution 1 day old. Carter's Antidote used for the other animals was 2 weeks old. The hypophosphite-hydrogen peroxide solution was 4 weeks old.

Rabbit	Weight	$HgCl_2$ 1%	Carter's Antidote	Lived		Necropsy Lesions	
						Local	General
32	1560	6.2	6.2	100 days			
111	1532	6.1	6.1	2 "		Marked	Slight
113	1473	5.9	5.9	4 "		"	Marked
116	1853	7.4	7.4	4 "		"	"
118	1210	4.8	4.8	1 day		"	"
121	1153	4.6	4.6	3 days		"	"
			Sod. Hypo-Phosphite 10%	Sol. of Hydrogen Peroxide ¼ Vol.			
31	980	3.9	3.9	2.0	3 days	Marked	Marked
109	1504	6.0	6.0	1.5	100 "		
122	1093	4.4	4.4	1.1	3 "	Marked	Marked
123	1333	5.3	5.3	1.3	6 "	"	"
125	1331	5.3	5.3	1.3	100 "		
126	1754	7.0	7.0	1.8	1 day	Marked	Marked

It is evident that Carter's Antidote in solution does not keep well, while the keeping qualities in case of the hypophosphite-peroxide mixture are unexpectedly good: for the average lethal period, in the latter case, was 35.5 days; and the percentage of survivals 33⅓.

greater diuretic action, seems to annul the antidotal value of the phosphite. Both of these series are too small to admit of final conclusions. Nevertheless they suggest that sodium acetate in certain proportions might act by introducing a condition of chemical instability which favors the occurrence of the antidotal reaction. An additional observation points in the same direction (Table VI).

The first half of this table shows that, when a solution of Carter's Antidote is kept for two weeks, it seems to lose its antidotal value. Only the first animal (No. 32), which received as antidote a solution 1 day old, recovered.

There apparently occurs a deterioration of effect on prolonged keeping of this solution, which might be due to rearrangement of ions, with production of a less active complex. Inasmuch as Carter's Antidote may be kept mixed, in the dry state (even in tablet form), this instability of the solution is of little practical consequence.

SODIUM HYPOPHOSPHITE AND HYDROGEN PEROXIDE.

This combination was first used, as antidote to mercuric chloride, in our previously published work on this subject.[1] Our reason for using it was the fact that the addition of hydrogen peroxide appreciably accelerated, in test tube experiments, the reduction of mercuric chloride by hypophosphite. That it has antidotal value, even when administered five minutes after the poison, may be seen from Table VII.

TABLE VII.

LETHAL DOSE OF MERCURIC CHLORIDE (0.04 GM. PER KG.) FOLLOWED 5 MINUTES LATER BY SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) AND VARYING PROPORTIONS OF SOLUTION OF HYDROGEN PEROXIDE.

Rabbit	Weight	$HgCl_2$ 1%	Sod. Hypophosphite 10%	Sol. of Hydrogen Peroxide ¼ Vol.	Lived	Necropsy Lesions	
						Local	General
10	1065	4.3	4.3	1.1	100 days		
11	1620	6.4	6.4	1.6	77 "	Marked	Marked
12	1700	6.8	6.8	1.7	6 "	Slight	Marked
19	1367	5.5	5.5	1.4	4 "	Marked	Marked
20	1780	7.1	7.1	1.8	4 "	"	"
21	1360	5.4	5.4	1.4	5 "	"	"
61	1568	6.2	6.2	1.5	2 "	"	"
62	1060	4.2	4.2	1.1	100 "		
				½ Vol.			
7	1330	5.3	5.3	2.7	25 days	Marked	Slight
8	1160	4.6	4.6	2.3	32 "	Slight	"
9	1370	5.4	5.4	2.7	6 "	Marked	"
22	1525	6.1	6.1	3.0	100 "		
23	1030	4.1	4.1	2.0	5 "	Marked	Marked
24	1020	4.0	4.0	2.0	4 "	"	"
				= Vol.			
25	1390	5.5	5.5	5.5	31 days	Necropsy Lost	
26	1200	4.8	4.8	4.8	5 "	Marked	Marked
27	970	3.8	3.8	3.8	4 "	"	"
				2X Vol.			
28	1490	5.9	5.9	11.8	100 days		
29	1390	5.5	5.5	11.0	4 "	Marked	Marked
30	1395	5.5	5.5	11.0	21 "	"	"
54	945	3.7	3.7	7.4	100 "		
63	986	3.9	3.9	7.8	5 "	Marked	Marked
64	1195	4.8	4.8	9.6	29 "	"	"

Sodium hypophosphite and hydrogen peroxide have antidotal value. Average lethal period 33⅓ days. Five animals among 23 (22%) survived 100 days.

To determine which proportion is best, we used varying proportions of hydrogen peroxide, with results the significance of which we are at present unable to explain. It will be seen that, when equal volumes of hydrogen peroxide and 10 per cent solution of sodium hypophosphite are used, there is hardly any antidotal action as is shown in the three cases in which it was used. We obtained the same result with two animals in our previous series. The series is, however, still too small to rule out the possibility of accident. With the other proportions, antidotal results were obtained; the best with twice the volume, the next best with one-fourth the volume. Summarizing all the experiments, the hypophosphite combination seems inferior to Carter's Antidote, the percentage of survival being 22 with the hypophosphite combinations against 33 with the phosphite mixture. The average lethal period was lengthened to 33⅓ days by hypophosphite against 44½ days of phosphite antidote. If on the other hand, we take the result of the best proportion, namely with two times the volume of hydrogen peroxide, the results are exactly the same. 33⅓ per cent recovery and 43⅙ days average fatal period.

It may seem strange that the combination of an oxidizing and a reducing agent should be of advantage in a reducing reaction. A thought that forces itself upon one is the suspicion that the hypophosphite might have been oxidized to phosphite by the hydrogen peroxide, and that the efficiency of the combination depended upon phosphite rather than hypophosphite. That this is not the case, can be shown by means of calcium chloride solution, which precipitates with sodium phosphite—calcium phosphite being insoluble—but which does not precipitate with sodium hypophosphite, owing to solubility of calcium hypophosphite. When we test our previously carefully neutralized antidotal mixture with calcium chloride, we obtain no precipitate, even though the hypophosphite-hydrogen peroxide mixture be previously boiled or be many weeks old. That these two agents do not seem to enter into reaction with each other, on standing, is further borne out by our rather surprising result with old antidotal solutions (Table VI), from which it will be seen that practically the same result was obtained as with fresh solution.

Further experiments are required to elucidate the reason for the efficiency of these combinations. To refer to them as instances of catalytic action would not add much, as we know so little about the real nature of catalysis.

CONCLUSIONS.

1. Carter's Antidote has given the best results in this study.
2. The efficiency of a mixture of sodium hypophosphite with hydrogen peroxide is but little inferior, and, in certain proportions, equal to Carter's Antidote.

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[2]Carter, Thomas A.: Chicago Med. Recorder, 1914, xxxvi, 444.

LABORATORY METHODS

APPARATUS FOR STUDYING THE EFFECT OF DRUGS ON THE ISOLATED GUINEA PIG UTERUS*

By Charles R. Eckler, M.S., Indianapolis, Ind.

THOSE who have occasion to study the effect of drugs on isolated organs suspended in Ringer's or Locke's solution, such as strips of intestine or the various structures from the male and female genital tracts, and particularly those who are engaged in the assay of ergot and pituitary products on the isolated guinea pig uterus, may be interested in a form of apparatus, here described, which has proved very convenient and useful for such purposes.

This apparatus was fashioned somewhat after that described by Dale and Laidlaw,[1] and in its original form, was first set up and used in this laboratory by W. F. Baker. It differs from similar apparatus chiefly in being practically all glass. During the three years or more in which the apparatus has been in almost constant use, several changes and additions have been made that seem to be decided improvements.

In its present form (Fig. 1), the apparatus consists of two units, each unit being complete by itself for the carrying out of an experiment. With the two units an assay of pituitary extract, for example, can be carried out in duplicate, or, two samples can be compared with a standard,—one sample with the standard in one unit, and the other sample with the standard in the other unit. And with this duplicate form of apparatus both horns of a uterus may be utilized,—one in one unit and one in the other.

The essential part of each unit is a water bath with included coils, tubes, etc. The water bath (Fig. 2-*1*) consists of a bell glass which is inverted and set in a large ring on a heavy ring stand. This bell glass measures 15 cm. wide by 20 cm. deep, inside, and has a capacity of about three liters after the other parts are in place. In its inverted position, the bell glass has one tubulature near the top (*3*), and one at the bottom (*2*). The top, or large opening, of the bell glass is covered with a disc of sheet metal—German silver (*4*), through which pass the various tubes, etc. The tubulatures are closed by rubber stoppers (*5* and *5a*). The bath is filled with distilled water to a height regulated by an overflow tube (*6*). Another tube (*6a*), connected with the overflow tube, allows the bath to be drained.

The water in the bell glass is kept warm by means of a tubular form carbon lamp (*7*). The size of lamp needed will depend on the room temperature, but 30 watt lamps have been used almost entirely. The lamp can be immersed full length in the bath or only part way, as necessary. When about the right

*From the Department of Experimental Medicine, Eli Lilly & Company.
1Dale and Laidlaw: Jour. Pharm. and Exper. Therap., 1912-1913, No. 4, 75.

depth is found, the room temperature remaining constant, very little attention is required to keep the temperature of the bath, indicated by thermometer *32*, practically constant. In order to maintain an even temperature throughout the bath, the water is kept continually circulating by gently passing through it a current of air which is conveyed to the bottom of the bath by a glass tube (*8*). In order to exclude the light of the lamp from the uterus or other material used, the lamp is painted black.

Two glass coils are set in the bath, the outer one (*9*), having a capacity of 200 c.c., is for the purpose of warming the Locke's solution which is allowed to flow into it from an elevated container,—a 2 liter aspirating bottle. By this

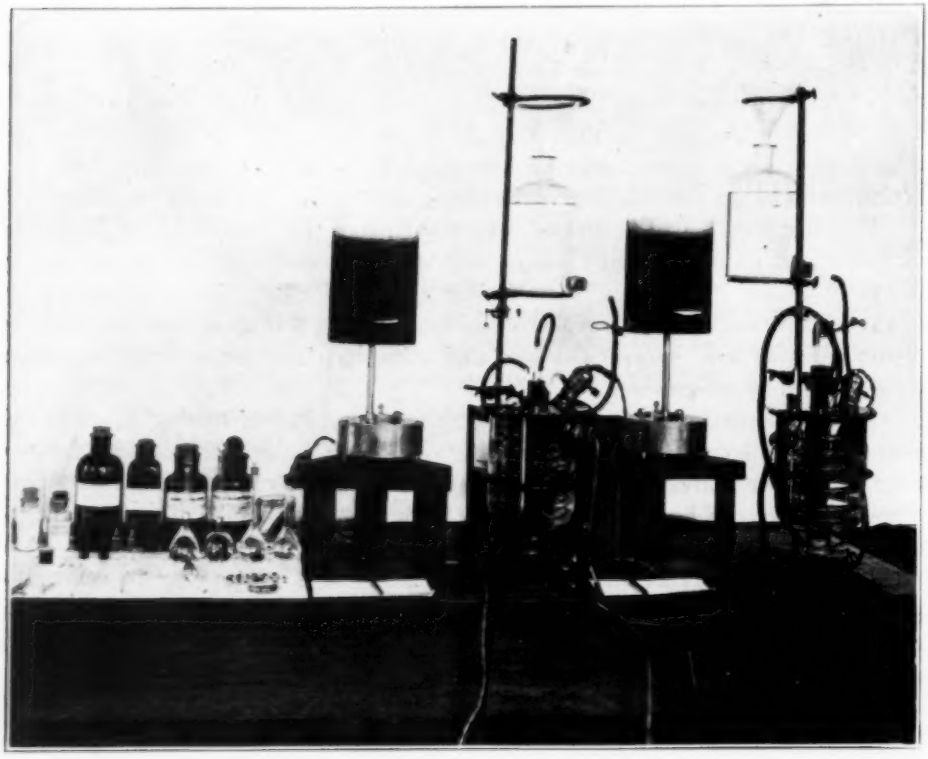


Fig. 1.

method of warming the Locke's solution in small quantities only as needed, precipitation of calcium salt is avoided which would to some extent occur if the whole container of solution were kept warm for some time. The lower end of this coil is joined to a tube forming a T (*10*), one arm of which (*11*) passes up through the lower stopper of an amber chamber (*14*), and the other arm (*12*) passes down through the stopper at the bottom of the bell glass. By means of a pinchcock at the elevated container (Fig. 1) and the pinchcock *13*, the Locke's solution may be flowed down through the coil and either up into the chamber by way of tube *11*, or down to a waste bucket by way of tube *12*, and, of course, the chamber may be emptied by way of tubes *11* and *12*.

The inner coil of the bath (*15*) is for the purpose of warming the air or

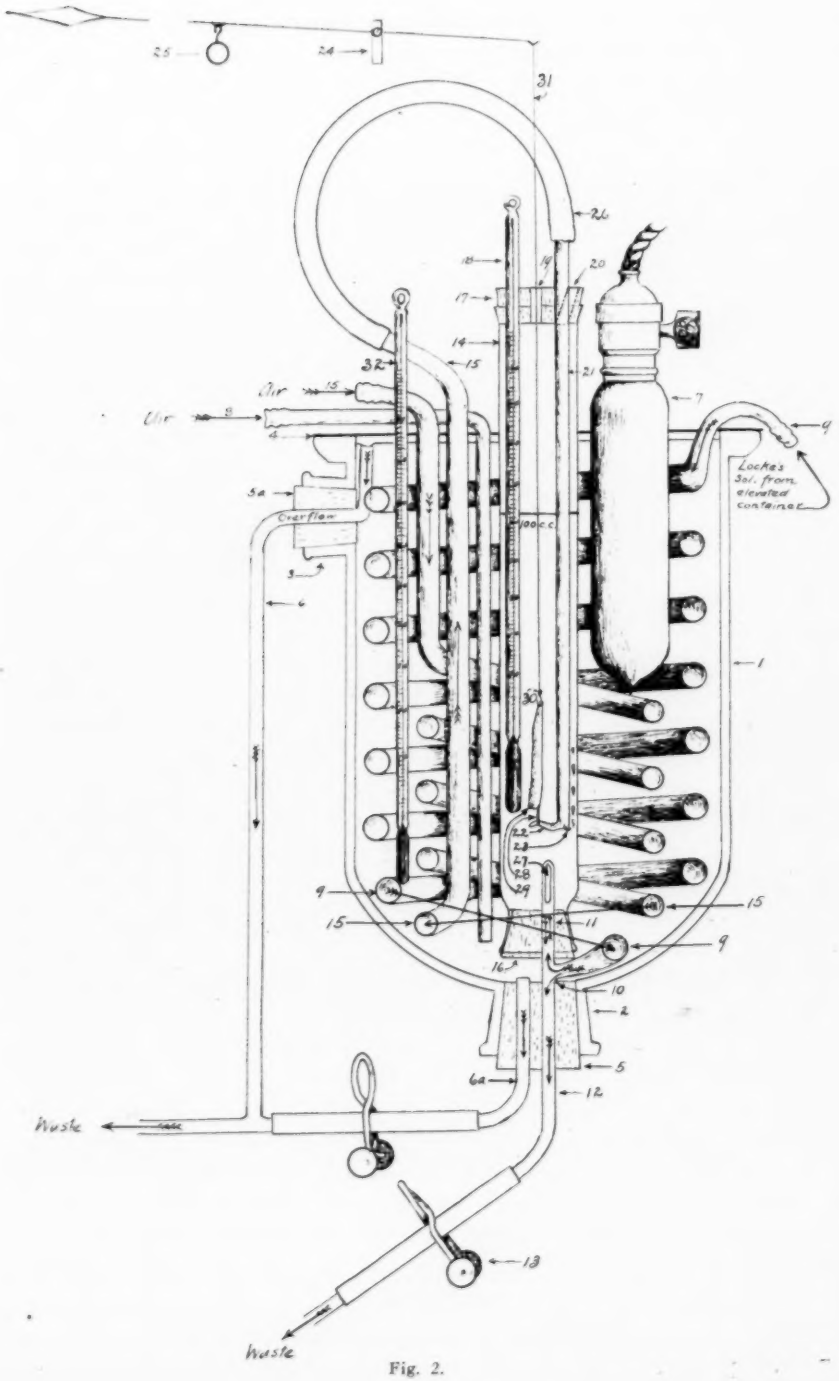

25
24
31
26
18
19
20
17
14
15
21
32
Air
15
Air
8
4
7
9
Locke's Sol. from elevated container
5a
Overflow
100 c.c.
3
6
1
30
22
23
27
28
29
9
15
15
11
9
16
10
2
5
6a
12
Waste
13
Waste

Fig. 2.

oxygen which is to bubble through the Locke's solution in the chamber. The main object of warming the air is to reduce the stimulation which the cool air would give to the uterus in passing about it when the chamber is momentarily emptied. Each time the chamber is emptied, one may stop the air from coming to the apparatus by pinching the rubber air tube, but this is somewhat inconvenient when two pinchcocks, in addition, have to be operated quickly. Managing any way one may, it seems that the emptying of the chamber will surely cause slight stimulation of the uterus, which may result in very slightly increased contractions. During the changing of the solution, however, one may stop the drum and if the writing lever or clamp holding it can be adjusted by a thumb screw, the writing point may be drawn away from the drum, and thus the record will not be disfigured by such added contractions.

The chamber (*14*) for holding the Locke's solution, in which the uterus or other organ is immersed, is made of amber glass tubing (3 cm. inside diameter) and is graduated to 100 c.c. It bears a cork stopper at the top and a rubber one at the bottom. As previously mentioned, the chamber is filled through tube *11* from coil *9*, and is emptied through tubes *11* and *12*, the solution flowing off to a waste bucket or drain. Tube *11* has a blind end in the chamber but is slotted on two sides (*27*) to prevent the current of Locke's solution from striking directly against the uterus when the chamber is filled. The stopper at the top of the chamber holds a thermometer (*18*) and a tube conveying air or oxygen to the Locke's solution. This stopper also has two holes, one (*19*) for the thread (*31*) from the uterus to the writing lever, and another (*20*) through which the pituitary solution or other drug is introduced.

The introduction of the drug may conveniently be made with a Record syringe having a long needle by means of which the drug can be injected directly into the Locke's solution. The air tube *21* has a small post about 1.5 cm. long near the bottom (*22*) to which the uterus (*29*) is attached, and the opposite side of the tube is drawn out into a little point or jet (*23*) from which the air escapes into the Locke's solution. The tube receives the air from coil *15*, which in turn receives it from the tank or compressed air system of the building. Coil *15* is connected with tube *21* by a rubber tube (*26*).

The thread from the uterus is attached to one arm of a counterbalanced Harvard heart lever (*24*), the other arm being weighted down (1 to 4 gm.) by hanging upon it small lead weights (*25*). Harvard slow-speed, single kymographs are used. These instruments are made to turn the drum once in ten hours, but for this purpose are speeded up to about one revolution in eight hours by diminishing the weight of the pendulums.

For the purpose of giving a clear understanding of the operation and use of this apparatus, the testing of pituitary extract will be very briefly outlined. (A somewhat more detailed description of the method is given in another paper.[2])

MATERIALS.

1. Chemically pure salts necessary for making Locke's solution.
2. Distilled water.
3. Pituitary extract to be tested.
4. β-imidazolylethylamine hydrochloride, or standard pituitary substance.
5. The uteri of 175 to 250 gram virgin guinea pigs.

[2]Eckler, Charles R.: Am. Jour. Pharm., 1917, lxxxix, No. 5, 195.

PROCEDURE.

The distilled water is warmed to 39° C. and introduced into the bell glass. The lamps are turned on and adjusted to the proper depth to maintain the desired temperature. While this adjusting is going on, the Locke's solution may be made up and introduced into the elevated chamber, the dilutions of the extract to be tested and the standard may be made, the drums smoked, the needles

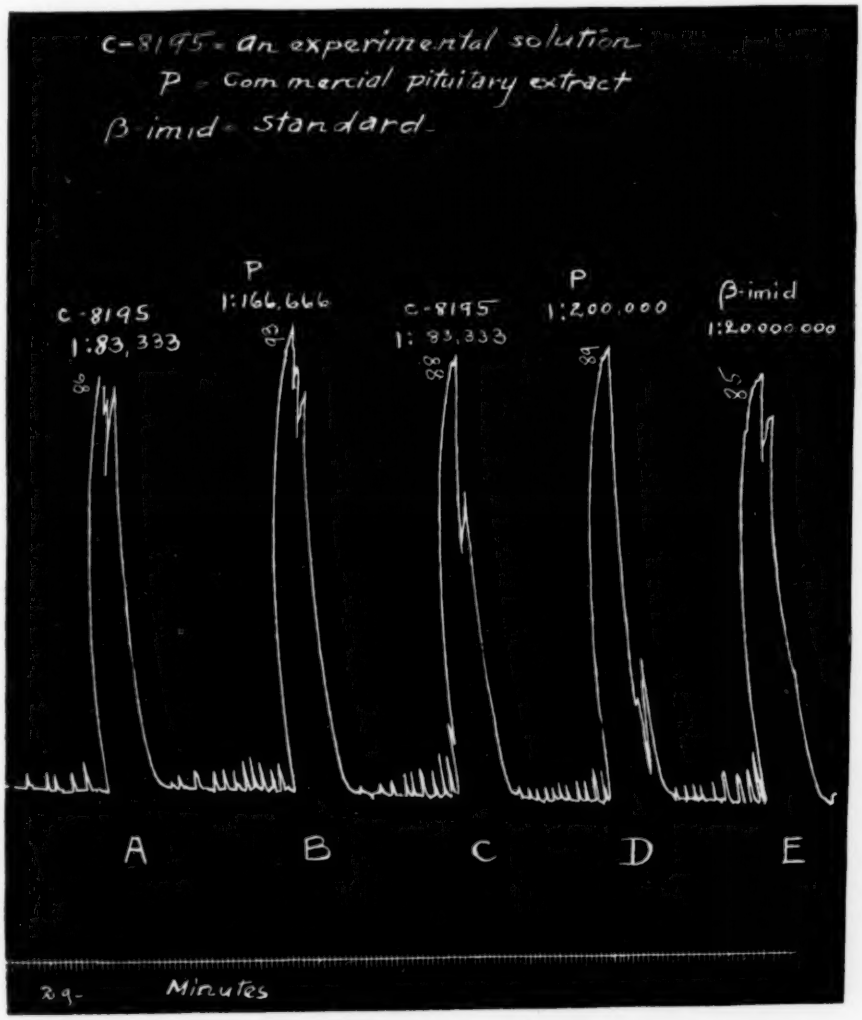


Fig. 3.

threaded, etc. When the apparatus is in readiness, the pig is decapitated, the entire uterus with Fallopian tubes and section of the vagina removed to a fold of cotton saturated with warmed Locke's solution, and the horns of the uterus separated, excluding the section of vagina. The vaginal end of a horn is tied to the little post on the air tube (*22*) by a silk thread (*28*) which is sewed into the peritoneal covering on the side of the broad ligament. The ovarian end of

the horn is attached to the thread (*31*) running to the writing lever by a small pin hook (*30*) which is passed either through the Fallopian tube or through the peritoneal covering as before.

The writing lever is weighted, and with a thumb-screw adjustment, the writing point is brought to the drum and the drum started. As soon as spontaneous movements of the uterus have appeared, which will usually be in about half an hour, the application of the standard substance is begun. It is first determined whether or not the uterus will react in a quantitative manner, and if so, the standard and unknown are then given alternately until a dilution of each is found which will produce like contractions of the uterus. For example, it may be found that a 1:200,000 dilution of the pituitary extract will cause approximately the same extent of contraction as a 1:20,000,000 dilution of β-imidazolylethylamine hydrochloride. (*D* and *E* on kymograph, Fig. 3).

The substances to be tested are carefully measured out with narrow pipettes and introduced into the 100 c.c. chamber with a long-needled Record syringe, in such strength of solution that the final dilution in the chamber will bring the substances to the dilution desired. For example, if it is desired to apply a 1:250,000 dilution of the pituitary extract to the uterus, 0.4 c.c. of a 1:1,000 solution may be introduced into the 100 c.c. chamber and the fluid, which will then measure about 99 c.c., made up to mark by rinsing the syringe and dose cup with sufficient warm Locke's solution, thereby diluting the 1:1,000 dilution 250 times. Or, if it is desired to apply a 1:20,000,000 dilution of β-imid., 0.5 c.c. of a 1:100,000 solution may be introduced into the 100 c.c. chamber and the fluid made up to mark as before, thereby diluting the 1:100,000 solution 200 times. The bubbling of the air through the Locke's solution equally distributes the injected solution in a few seconds.

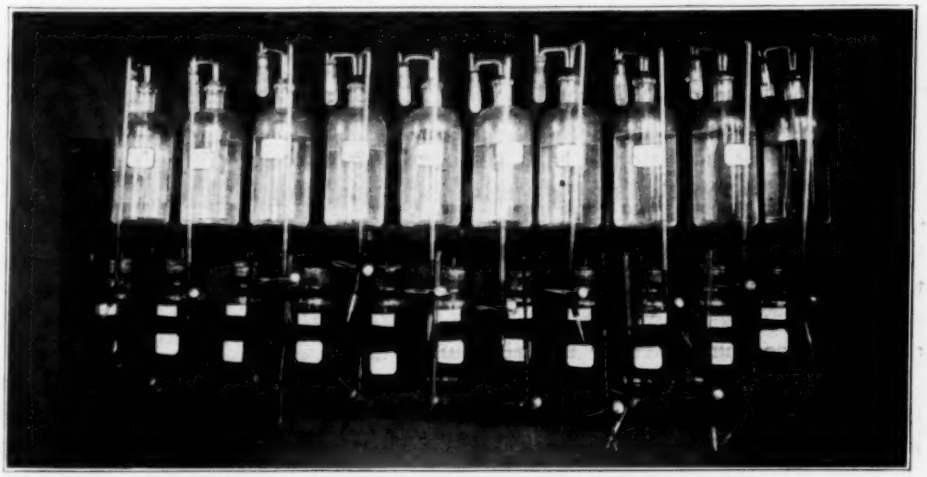
After the application has been made and the writing point has registered the greatest contraction the uterus will make with the given dilution of drug, which will usually require from three to five minutes, the chamber is emptied by opening the pinchcock *13*, when the fluid will flow out through tubes *11* and *12*. Closing this pinchcock and opening the one at the elevated container, Locke's solution will flow in from coil *9* and fill the chamber. In order to remove all considerable traces of the drug which may have adhered to the sides of the chamber or diffused from the wall of the uterus, the chamber is emptied again and refilled to within 1 or 2 c.c. of the 100 c.c. mark. After about 15 to 30 minutes, depending on the activity of the particular uterus, another application of drug is made, and so on, until the end result is reached.

THE USE OF INDICATORS TO DETERMINE H-ION CONCENTRATION—APPARATUS FOR TEACHING*

By Louis Rosenberg, B.A., Dallas, Texas.

WITH very little modification the following introduction from Professor Carmichael's address on Mathematics in the May 18th issue of *Science* applies to chemistry in the medical curriculum: "Mathematics beyond the merest elements has been regarded by some as an excrescent malady by the human spirit, generated like the pearl in an abnormal and morbid way and representing a nonliving embedment in the active tissue of the organism of society; by others it has been supposed to exhibit the highest intellectual reach of mankind, being in itself the most powerful tool yet devised for the interpretation of natural phenomena, while at the same time it affords a satisfying expression of the furthermost esthetic attainment. On the one hand, it is considered a piece of jugglery in which it is the joy of the proficient to produce more and more complicated entanglements to astonish the beholder and overwhelm him with the sense of mystery; on the other hand, it is seen to be the systematic unfolding of remarkable and important properties of a highly fascinating creation or construction of the human spirit by means of which it has at once its most intellectual delight and the best means of understanding its environment. . . . . But it is true, I believe, that mathematics is generally recognized as essential at least to scientific progress."

I take it therefore that those who are striving to teach the chemistry that modern medical students should learn have met the same difficulties as the writer: woefully deficient preliminary training in the science, absolute lack of interest, forced and inefficient application and plenty of charitable advice by well-meaning but ill-advised practitioners that *their* chemical courses were a



*From the Department of Chemistry, Baylor University Medical College, Dallas, Texas.

joke, that they never used chemistry in their practice and that it is more than a nuisance in the medical curriculum.

I believe, therefore, that you would welcome any suggestion that will facilitate the teaching of any concept that we believe of fundamental value in the equipment of the modern medical student and I submit herewith a device designed and made by the writer which has proved invaluable in the teaching of the subject of indicators and their use to determine H-ion concentration.

Referring to the cut it is seen that the apparatus consists essentially of a portable rack having two shelves: the upper carries one liter bottles containing solutions of H-ion concentrations unknown to the student; the lower shelf carries the indicators arranged in a series of descending H-ion reaction. The solutions are prepared according to the chart on page 157 of Hawk's Practical Physiological Chemistry; the indicators are selected from the lists in the above text and in Mathews' Physiological Chemistry. I have found the following series of indicators best: Nauvein, Tropaeolin 00, Methyl Orange, Methyl Red, Congo Red, p-Nitro-Phenol, Rosolic Acid, Tropaeolin 000, Phenolphthalein, Thymolphthalein and Tropaeolin 0.

The general design and arrangement are clearly shown in the cut. The only feature requiring comment perhaps is that by which evaporation of the solutions in the top row of bottles is prevented. At first a mercury valve was employed but this proved unsatisfactory. The arrangement shown can be made very easily by sealing off small test tubes to the desired length and using small tubes drawn out to about a one millimeter capillary, thus to expose a minimal area of the solution in the test tube, which is of course the same as in the associated bottle.

The apparatus is inexpensive and can be made up, solutions and all, in a few days.

MASS URINALYSIS

By Mortimer Warren, M.D., New York City.

THE knowledge of the essential value of laboratory examinations, in diagnosis, is universal.

Independent laboratories have come into existence to furnish one of the means by which the physician can meet this demand of his practice. The laboratory has, in fact, assumed the responsibility for the performance of even those simple tests which compose a routine analysis of urine. The concentration of effort resulting from the separation of duties should lead to increased reliability of method and should furnish accurate information within the limits of the service undertaken by the laboratory.

The laboratory, however, tends to become completely disassociated from the stimulus of the problems of diagnosis. A laboratory thus constructed is, in essence of organization, a factory. It follows that the bulk of the work will be done by a technically trained staff whose labor in great part consists of monotonous repetition of routine procedures.

The system in vogue in life insurance laboratories in general emphasizes the specimen of urine as the unit. Here the object is to classify a given individual on the basis of the urinary findings. Casts, for example, are considered to be of so much significance that an exhaustive search of the sediment is required.

Another system which has certain advantages, when a large number of examinations are to be carried on, makes use of the individual test as the unit. So far as sediment is concerned, an average time limit can be found which will broadly cover the ground. The expression "Casts not found" then means that no casts were found in the time devoted to the search. My remarks are concerned with a personal experience in a laboratory which has conducted its work along the lines mentioned above. It has endeavored to standardize the methods and has subjected all specimens to the same routine with additions to suit indications. I believe it may be of interest to outline the methods in use and to discuss briefly the results obtained so far as they are accessible. The system is a composite which would naturally be developed in some form by anyone under similar conditions.

ROUTINE.

1. Color.
2. Reaction (litmus paper).
3. Specific gravity (room temperature).
4. Albumin and quantity if in sufficient amount.
5. Sugar and quantity if in sufficient amount.
6. Indican (when requested).
7. Bile pigments } Noted in color of urine and confirmed by test when presence is suggested by the color.
8. Blood pigments }
9. Urobilin (if color gives indication, or if requested).
10. Acetone bodies (ferric chloride test) (if requested or when sugar is present).
11. Urea (hypobromite method) (if requested).
12. Sediment.
    1. Epithelium—flat—round (small or large).
    2. Leucocytes—relative abundance or absence.
    3. Erythrocytes—relative abundance or absence.
    4. Crystals—character.
    5. Casts—relative abundance or absence—character.
    6. Bacteria—yeast, etc., and other elements.

PROCEDURE OF EXAMINATION.

Specimens are entered on successively numbered cards which follow the specimens as they are examined. Special requests or remarks are noted. The specimens are run through in sets of a size which varies according to daily circumstance. The bottles are arranged and numbered 1, 2, 3, etc., from left to right with a grease pencil, the accompanying cards being numbered to correspond. Other sets of the same day are numbered 1′, 2′, 3′, etc.

STEPS.—

A. Specific gravity—appearance—color—reaction.

B. The bottles are well shaken and the urine is poured into 15 c.c. centrifuge tubes in a rack of two rows, holding twenty-four in all. These tubes are

numbered 1, 2, 3, etc., from left to right. They are centrifugated for a specified time at a standard speed.

C. From the centrifugated tubes the supernatant fluid is carefully decanted from the visible sediment into test tubes in a rack for albumin, sugar, etc. The sediment in the centrifuge tubes is reserved for microscopic examination.

D. From rack C, always left to right, (1) a rack of 24 tubes is filled one-half full of urine for albumin; (2) to the 24 tubes of a copper rack, each of which contains Benedict's solution up to a certain mark (cross beam of rack), is added about one-sixth volume of each one of the specimens from rack C; (3) remainder of fluid in rack C is reserved for other examinations.

METHODS.—*Albumin.*—To each test tube of D^1 is added one-sixth volume of saturated sodium chloride solution ($NaCl_2$) C. P. and two or three drops of 3 per cent acetic acid. The upper portion is heated to boiling in a rack devised for the purpose, two or three drops of acetic acid are added and the upper layer is examined by means of a bull's-eye light against a black background. Presence of albumin is noted as +, ++, +++, and more than +++ is determined quantitively by Purdy's centrifugal method.

Treatment of Cloudy Urine.—To a test tube one-half full of urine is added 1 c.cm. of 10 per cent sodium hydrate; the urine is filtered,* the filtrate is acidified, and the usual albumin test is proceeded with as above.

Sugar.—Rack (D_2) is immersed in a boiling calcium chloride bath for two minutes (calcium chloride, technical—saturated watery solution) (Myers Hospital urinalysis). Those tubes which show reduction are noted and the finding is confirmed by a second test with Haines solution. In case of difficulty of interpretation, a phenylhydrazine test is done.

Quantitative Sugar (Adaptation of Benedict's Method).—Five c.c. of Benedict's reagent in a porcelain dish with one-half teaspoonful of calcium carbonate is placed over a flame. The urine is run in from a 10 c.c. Mohr pipette (urine diluted 1 to 10). A table for readings is constructed which gives twenty-one readings from 0.2 per cent to 10 per cent, varying in the lowest range by 0.2 per cent, in the medium by 0.5 per cent, and in the highest by 1 per cent. In this procedure it is taken for granted that every reducing substance which gives a positive phenylhydrazine reaction or which allows of a definite titration with Benedict's quantitative reagent is glucose. No attempt is made to separate the unusual carbohydrates.

Sediment.—Tubes of rack B are kept in this rack until examined. From each tube the sediment is carefully decanted so as to leave about ½ c.c. of residue. This is thoroughly shaken and deposited on a glass slide by tapping the upturned tube against the slide. An examination is made with low power of the entire drop; undifferentiated elements, if present, are examined with high power.

Aside from indican, other methods outlined in the routine require no ex-

*Centrifugation clears urine as well as filtration does. A cloud composed of urates dissolves with heat, one due to phosphates with acid. A cloud composed of bacteria will not be removed by filtration or by centrifugation; in this case the sodium hydrate method of clarification is used. Occasionally a trace of albumin will develop in these urines when not originally present, due apparently to bacterial proteins. The practical result of this procedure appears to be fairly reliable although the precipitation possibilities of albumin may be interfered with by the addition of alkali. (I am unaware of the source of this method.)

planation. The method in use for indican is Jaffe's, which consists of clearing urine with lead acetate, adding equal amounts of Obermeyer's reagent and 1 c.c. of chloroform. The test is time-consuming owing to the slowness of the reaction and thus the necessity for repeated periods of agitation and sedimentation. The mere presence of indican can hardly be called abnormal. Its determination should be supplemented by an estimation of the amount present. This is done by noting the number of drops of potassium chlorate solution (3.2 per cent aqueous) necessary to decolorize the indigo. The findings in certain urines may then have interpretative value though the specimen of urine is a single one.

DISCUSSION.

In order to arrive at any conclusive opinion of the value of such mass urinary examinations, it is necessary to consider what is to be expected:

1. The work should be reliable. Every notation should represent the result of definite procedures.

2. Abnormal elements should be detected and certain of them expressed quantitatively.

3. Expression of findings should be simple and direct. There should be no attempt to distort relative values or emphasize the importance of the work by means of estimations and calculations of a purely paper significance.

4. It should be understood that no evidence of a metabolic nature can be obtained from quantitative estimations of single specimens of urine. No specific diagnosis is possible except in rare instances such as Bence-Jones' proteinuria.

1. *Reliability of Work thus Conducted.*—In general, the character of the work is a direct reflection on the character of supervision. An intelligent, efficient, and conscientious staff is comparatively easy to assemble. Supervision covers outline of methods, distribution of labor, development of *esprit de corps* and sympathetic knowledge of the character of the work and of the relation of fatigue to objective accuracy.

This system, I believe, provides all one could ask in furnishing the information which may be derived from routine examinations. Its actual product is difficult to analyze. In an attempt to ascertain the character of results, specimens were sent through at various times in duplicate. In the series there was in one instance a variation of specific gravity of 3 points, failure to find a few hyaline and granular casts in another instance, a variation of 4 per cent in bulk albumin percentage in another. Otherwise, the findings were in agreement. To determine the personal element in albumin readings a series of forty-eight urines were read by five different individuals. In this series the maximum number of specimens without albumin was, according to one observer, 36, to another, 24. On the other hand, there was entire agreement on large amounts with shades of difference between so-called $++$ and $+++$. In other words, it is obvious that the shading of the nonalbuminous urine into that containing more or less definite amounts is such that absolute agreement can not be hoped for. In fact, the reported presence of a faint trace of albumin without confirmation or without other evidence on the clinical side has no significance. The finding of albumin or casts or both can not be interpreted by the laboratory.

Their importance (in case the finding is accidental) is simply to draw the closer attention of the physician to his patient.

2. *The Detection of Abnormal Elements.*—Bile, blood pigments, urobilin and other coloring matters would not be found unless in sufficient amount to attract the attention of the observer who notes the color, appearance, etc. Whether Bence-Jones' protein would be discovered or not I can not say by actual experience. In the only case which I have seen, this was overlooked in the routine examination of urine in a hospital. The routine method here outlined, using a rack and heating several urines at a time, would, I believe, offer less chance for the error than the hurried individual test where one, unless expectant, is likely, owing to the early precipitation of the protein, to shorten the necessary period of actual boiling.

Paragraphs 3 and 4 are obvious statements.

CONCLUSION.

I believe this system fairly meets the requirements, allowing for a small percentage of error. I do, however, believe it should develop greater accuracy. It lacks the element of confirmation. This can readily be obtained by duplicate examinations in the case of description, specific gravity, albumin and sugar. The two latter tests should be repeated for exactly the same reason that no one depends upon a Wassermann reaction which relies on a single test and its control. Though the importance is less, the principle is the same. An approximately accurate determination of the specific gravity is important from a functional point of view when the fluid intake of the patient is under control. There is special liability in "piece work" to read the urinometer before it has come to rest under proper conditions.

The description of urine, its color and appearance have an important bearing on the subsequent examination and therefore can not be too carefuily noted. In conducting these confirmatory tests, a second portion of centrifugated urine would be furnished which would allow of a second examination in cases where there is reason to believe the particular drop under observation is not representative.

The collection and preservation of urine are important factors. Under the conditions which I have described, in the summer months a large proportion of the urines examined are so contaminated that the microscopic examination is of no value and represents a lot of useless labor. Urine should be properly collected. It should, so far as is practicable, represent the kidneys, bladder, and urethra, and not the prepuce and vagina. The urine should be collected in a scrupulously cleaned vessel and immediately delivered into a sterilized bottle, or where possible, passed into the bottle, after thorough cleansing of the parts. Preservation of urine is unsatisfactory in my experience. Boric acid, which is the only practicable substance on account of its noninterference with the usual tests, is only mildly antiputrefactive; but I believe it would have a much better chance if the above precautions were used in collection.

Centrifuge tubes should be cleaned with soap and water, then with weak acid and thoroughly rinsed, drained, dried, and sterilized with hot air. All test tubes should be put through the same process; there is, however, no need

of sterilization. Centrifuge tubes require sterilization in order to avoid as much as is possible the rapid deterioration of urine which takes place in the moist air of the laboratory in the period which may elapse between centrifugation and examination.

GUINEA PIGS FOR PROFIT

By M. A. Kroeber, Mt. Vernon, N. Y.

ONE finds it easy to do what one can do well. This applies to raising guinea pigs as to other things; and it is easy to do it well for guinea pigs because they give satisfactory returns for simple care. With proper food at regular intervals and clean cages the caretaker does not need to know much about causes of death or treatment of disease.

The guinea pig's only enemies, if kept in the house, are rats, but outdoor runs must be protected from cats and dogs.

To have recognition in supplying hospitals with animals the breeder must be able to raise them in large numbers so as to continually reserve a sufficient number from which to breed so that the stock may not diminish toward spring. The laboratories make small, practically no demands for animals during the summer months, so that in September the supply is in excess of the demand. By February, however, there are not so many on the market, and by May there is a shortage among the dealers. This causes a temptation for the breeder to sell more in March and the following months than is consistent with reserving stock for the fall.

Some hospitals use animals as small as ten ounces, others stipulate at least five hundred grammes. To raise an animal to the latter weight consumes six months. All circumstances being favorable, they may reach that condition somewhat before, but adverse circumstances may easily prevent. Among the causes which deter the rapid attainment of weight is premature conception, animals not being in a condition for mating under six months of age and eight months is distinctly advantageous, for a mature mother shows no ill effects from bearing young and makes a rapid resumption of strength for another healthy litter of large animals. If mature animals are used to breed from, there are no abortions and full litters of well-developed young are produced. When mothers are too young the first litter may cause the death of the mother, or though the first may be healthy, subsequent litters are usually spindly and inclined to still-births. First litters do not number as many animals at later litters, one or two being usual. The mother thereafter produces three to five, more being out of the ordinary and undesirable from the standpoint of vigor.

In order to prevent animals from conceiving prematurely, the sexes must be separated at the age of one month, when the young are taken from the mother. A number of small animals may be kept to advantage in the same cage if of an approximate age. If large and small animals are kept together the weaker are trampled upon and are not allowed sufficient food. As the animals approach six

months of age the males develop decidedly pugilistic tendencies which makes it desirable to sell them early, keeping only necessary mates. The females are not prone to fight until after successive families when they lose their smoothness of temper. It is best to have only two mothers in a cage together at the time of birth and nursing.

Six or even eight females can be allowed to a male at a time, but they do better if they are separated from the mate when in kindle about a month. They carry their young two months and nurse them from three to four weeks. Four litters, therefore, is the most one can raise from a female in a year, a good mother often having only three—ten to twelve babies a year in all. A good breeder in her prime produces twenty-two or twenty-four young with neither still-birth nor abortion. She should not breed after three years of age, and three years is the outside limit of desirability for sale. With good care they do live three or four years longer as pets.

Oats and hay with abundant green food keep guinea pigs in the pink of condition, the succulent food being a laxative without which they do not thrive, while if the oats are omitted they fail in attaining good weight in short time. Bread is eaten by some but is not economical; neither is grass advantageous in the diet if one aims to get rapid results.

A healthy animal is sleek of fur, clear of eye, and cheerful—moping being a sure sign of sickness. The animals thrive best in a temperature not under fifty degrees, which puts draughty barns out of the question as quarters in winter. They can stand a cool habitation but if exposed to draught or wet bedding succumb to pneumonia.

Sawdust or hay make the best bedding, preferably the former, and an insecticide is never needed if the cracks and corners of the cages are kept clean.

If an animal is ill from cold or injudicious feeding two or three drops of castor-oil can be given to advantage and the dose repeated if necessary. For loose bowels omit the green food for two or three days or for constipation supply greens in abundance—clover, dandelion, lettuce, celery, beet-tops, carrots or carrot tops, etc.

The floor of cages should be impervious to moisture, either of zinc, galvanized iron, or of wood treated with a moisture-, acid-, alkali-proof paint. Ventilation should be good, fresh air being absolutely necessary. An escape of illuminating gas is fatal.

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EDITORIALS

The Physician's Responsibility in Connection With the Food Situation

EVEN prior to the present unusual economic conditions created by the war for democracy, cries of the increased cost of living were heard on every side. Much of this increase, particularly in the case of food, has been due not so much to any real scarcity in the staples of life as to the development of more luxurious habits of living. These concern not alone the particular foodstuffs, but the manner of their delivery to the consumer; elaborate retail stores in expensive buildings, the use of fancy and unnecessary packages and containers, and insistence on prompt delivery, have all contributed toward raising the retail price of food far above its real value. The trouble with us has been not so much the increased cost of living as the cost of increased ease and elegance of living. The wealth of the country has been so great that the consequences of extravagance were not felt by the people as a whole; but now we face the possibility of a real shortage of food—at least what would be one were the standards of the past few years insisted upon. To avoid such a catastrophe we must recast our ways of living; we must learn how to secure an ample

sufficiency of nutriment for even the poorest of our people from other sources of foodstuffs than those that we have been accustomed to use. No one who is familiar with the actual state of affairs has even hinted at the possibility of an insufficiency of nourishment for every individual in this country, provided there is no waste and the dietaries are so adjusted as to use those foodstuffs that are available. There is no danger of a food shortage if the available supply is properly used, but such might easily develop and a state of partial famine become established, did we continue to live by our old wasteful standards.

The greater part of this adjustment can be made by centralized administrative power, but this alone may prove inadequate, and the administration be seriously embarrassed in its work by popular disapproval and misunderstanding, unless the actual consumer comprehends the principles upon which such control must, and no doubt will be, based.

Elsewhere in this Journal is published an article dealing in an elementary way with the principles upon which control of diet is based. It is thoroughly appreciated that to the majority of the readers of the Journal many parts of such an article will appear to be superfluous, because they deal with elementary truths of dietetic physiology which every physician knows or used to know, but it is hoped that placing the newer less familiar pieces of knowledge in their proper relationship with the old will be considered as sufficient justification for adopting such a method.

In the readjustment of diet which each family must probably make during the ensuing year, it will frequently be necessary for the physician to see that no serious mistakes leading to under-nutrition have been made. He will no doubt be consulted by his patients on this particular point, and it can not but help him in giving good advice if he avails himself of an opportunity to rub off some of the rust which from disuse must have developed on the machine of dietetic physiology and hygiene which as a student he was more or less familiar with. The old machine has, moreover, become much changed and added to in recent years.

The average diet used by the individuals of a community depends on a variety of circumstances. For practical purposes these may be divided into two groups, of which the one—the economic—concerns the easiest, most convenient way of supplying the food, and the second—the physiological or hygienic or personal—concerns the requirements of the body for food. It is particularly with the second of these that the housewife can be of incalculable service if she places herself in a position rightly to adjust the diet so as not to interfere with its food value. If the economic situation should make it necessary that changes be made in the amount and nature of the food allowed each individual, it will rest with the housewife to adjust her table to the new requirements without interfering with the efficiency of the diet. In order to do this she must have some means for measuring the adequacy of food—some value or standard by which she may appraise its true nutritive worth. If, for example, the available supplies should demand that each individual must replace in his diet some foodstuff, such as wheat flour, to the use of which he has become accustomed, with other less familiar foodstuffs, how are we to know how much of the one should be used to replace the other? Unless the substitution be in-

telligently made, serious disturbances to health are very likely to follow, especially in the case of the young and growing members of the household.

In the present conflict between autocracy and democracy in which our country is engaged, the one thing upon which victory will rest is efficiency—efficiency not alone of those who are fighting for us on the battlefields and of the administrative authorities of our army and navy, but efficiency of the people at home. In no way can this domestic efficiency with regard to the problem of the adequate and intelligent household administration of food display more practical effect than now at the very outset of this country's entry into the war. By carrying scientific efficiency into every detail of our living, we can beat the Germans at their own game.

The object of the present article is to explain the standards upon which this control of diet depends. It is not intended to prescribe in detail what foodstuffs should go to make up a particular dietary, for this must vary with the requirements, the individual tastes, and the purse of the family, but to explain in simple language the principles upon which such details can be worked out. To present you with innumerable diet sheets for every conceivable condition that might arise, would not only be confusing, but as useless in instructing one in the principles of dietetics as it would be to insist on a medical student's learning by heart the composition of a medicine for each disease he might meet with in his practice. We do not attempt to do so, but we make him study the principles upon which he may himself compose his medicine to suit the peculiar symptoms of each particular case.

—*J. J. R. M.*

The Occurrence of a Lymphocytosis as One of the Late Symptoms of Gas Poisoning

A PECULIAR and striking change in the lymphocyte cell count of the blood in patients who have suffered from gas poisoning has recently been observed by Miller[1] and Rainy.[2] This change consists in a very marked increase, both relative and absolute, in the number of lymphocytes. It would appear that this increase develops only after a period of a month or six weeks following severe gas poisoning, or after a somewhat longer period if the intoxication was of a milder degree. And in cases with persistent symptoms the lymphocytosis may continue a long time, at least for eighteen months.

The type of cells which is increased is the small lymphocyte with relatively large deeply staining nucleus and relatively little protoplasm. In a few cases there was a fair sprinkling of larger lymphocytes with a broader rim of protoplasm and a larger nucleus. No other type of blood cell appeared to be influenced in any constant fashion.

In a series of normal persons the average number of polymorphs was about 70 per cent, while the lymphocytes constituted about 20 per cent of the total white cell count. This was found in cases in which the total leucocyte count

[1]Miller, Capt. James, and Rainy, Capt. Harry: The Lancet, London, 1917, cxcii, Jan. 6, 19.
[2]Miller, Capt. James: Ibid., 1917, cxcii, May 26, 793.

varied from 5500 to 9000 per cubic millimeter. But in marked cases of gas poisoning it was found that when a general leucocytosis occurred there was also an absolute increase in the number of lymphocytes; for example, in one severe case with a total white cell count of 17,000 per cubic millimeter there were 6401 (37%) polymorphs and 10,034 (58%) of lymphocytes. Apparently in a relatively very small number of cases (usually mild ones) this phenomenon was scarcely noticeable, the number of lymphocytes being almost, if not quite, down to the normal limits. But its constancy in the great majority of cases in which symptoms persist is regarded as of particular significance, especially in the matter of diagnosis and in the estimation of the degree of severity of the injury produced by a previous exposure to a poisonous gas.

The question of the character of the gas which may produce these effects arises at once. On this point the information obtained was unfortunately very indefinite. The blood change would appear to have no relation to the nature of the gas employed. Undoubtedly some of the cases were due to chlorine, but others were evidently due to other forms of gas, and the conclusion is drawn by the observers that probably all the poisonous gases which have so far been extensively used in the war may produce this effect. On the basis of the late symptoms the cases could be roughly classified into three groups, viz., those in which gastric symptoms predominated, those in which respiratory disorders were more prominent (not infrequently both types were met with together), and finally cases which exhibited chiefly nervous symptoms. But apparently the character and origin of these symptoms in nowise influenced the extent and nature of the blood changes.

Lymphocytosis is a characteristic symptom in a considerable variety of pathological conditions. The authors have considered those conditions carefully in order to determine whether or not they could find any common feature or lesion which might also be present in patients suffering from the late effects of gas poisoning, and which might throw some light on the origin of the lymphocytosis. These considerations have led to the tentative conclusion that probably a chronic inflammatory change in the respiratory and gastric mucus membranes is at least a factor in the production of the blood changes.

It is further significant to note that after a long and careful study of many cases of gas poisoning the authors find it necessary to state, "we are still in the early stages of our knowledge of the effects of gas poisoning on the body."

—*D. E. J.*